THE
BACTERIOLOGY
OF
SURFACE WATERS
IN THE
TROPICS

W. W. CLEMESHA

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BY

WM. WESLEY CLEMESHA, M.D. (Vict.), D P.H

MAJOR, INDIAN MEDICAL SERVICE; SANITARY COMMISSIONER, BENGAL;
PROFESSOR OF HYGIENE, MEDICAL COLLEGE, CALCUTTA;
LATE OFFICIATING SANITARY COMMISSIONER, MADRAS

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PREFACE.

In the year 1909 an appendix to the annual report of the King Institute of Madras was published by myself and my assistant. This work described the commencement of our study on the bacteriology of drinking water-supplies in Madras and contained great deal of extremely interesting material collected over a period of about 12 months. Had it not been for the fact that my stay in Madras was rapidly drawing to a close, these results would certainly not have been published at the time they were; another year's work would have greatly increased the value of the report. The study of the bacteriology of drinking water has been continued both of the King Institute in Madras and in my own laboratory in Bengal for a period of about 4 years. Hence it appeared to us desirable to re-write the Madras report and to bring it up to date in the light of the experience gained recently.

As my assistants (Assistant-Surgeon T. Seethapathy Aiyar, L. M. & S., and V. Govindaraju Mudaliyar, B.A.) participated in the original report, they must be mentioned as co-workers in this small book. The latter of these gentlemen has subsequently come as my Head Assistant in my laboratory in Bengal and is largely responsible for the results reported in this book.

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CHAPTER I.

INTRODUCTORY.

In beginning the study of the bacteriology of water in a tropical climate, it must be perfectly apparent to everybody that natural conditions in the tropics are widely different from those in colder climates. In a thickly populated and small country like England very few really natural sources of supply could be used without some purification process. Large lakes, where they exist, are used as town supplies after suitable purification; but nobody would ever dream of using small ponds or lakes. Artificial storage reservoirs are common, but in these instances great care is taken to keep the collecting ground as free from pollution as possible, and even then careful filtration is always made use of.

As for the rivers, they are mere streams when compared with the great rivers of the East. The current is usually rapid, so that it cannot take more than a few days for any particular drop of water to pass from the sources to the sea. Practically all are highly polluted with large quantities of sewage, or effluent (which is bacteriologically the same thing), derived originally from human beings. The Irwell, the Lea, the Ribble, and many others that could be named, are little more than open sewers.

England has a cold, dull, grey climate with frequent rain and little sunlight in the year. Taking these facts into consideration, it is hardly to be expected that in England the natural purification of surface waters should have received much attention. From the point of view of drinking-water, England is a land of scientifically purified waters.

Let us turn to the other side of the picture. In the East, lakes, ponds, tanks play a very important part in the water-supply of the country. In many provinces the only available supply is from the village tank, which is nothing more or less than a pond of greater, or smaller, dimensions. The ancient rulers of this country recognised the advantages of large and well-constructed tanks, for in many districts large lakes have been built in order to store water both for drinking and irrigation purposes. Of course ground water, obtained from wells, is used where local conditions are favourable, but a large proportion of the inhabitants prefer lake or river water, if it is obtainable.

When one considers the rivers of the East, we appear to be dealing with a subject that has no parallel in the West, so great is the difference between the sewage-polluted streams of England and the mighty floods, such as the Ganges, the Indus, the Irrawaddy, the Mahanuddi, the Kistna, the Brahmaputra, and a hundred others that occur to one. All of these are many hundreds of miles in length ; they move slowly over a flat country ; at certain times of the year they broaden out into wide expanses of shallow clear water resting on a bed of clean sand. It is impossible to give any idea as to how long it would take for any particular drop of water to pass from the source of one of these leviathans to the sea. Towns of course exist on their banks, but comparatively few of these have an underground drainage system, or pipe water-supply, so that although the pollution may be very deadly in character, the quantity is so small that it is but as a drop in the ocean. The contamination occasioned by a large town, such as Benares, is relatively so small that it is not always easy to trace it a few miles down the river. Of course, along both banks, there is a constant trickle of contamination greater or smaller in amount in different places. Yet, in spite of the small amount of polluting material, we have to explain the fact that evidence of fæcal contamination can nearly always be found even in small quantities of water.

To turn to the climatic conditions, for 7 or 8 months of the year most of these rivers flow under an almost cloudless sky. The sun's rays are practically vertical, the amount of evaporation from the surface is great, the water is in many cases clear. So great may be the combined result of these natural forces that it is occasionally possible to obtain a sample from the middle of the Ganges in May, which will contain no glucose fermenting fæcal organism in 100 cubic centimetres.

Then again there is the monsoon period, during which these rivers become rapid flowing seas of muddy water. Under these conditions they more nearly resemble the conditions of Western streams, for six months' accumulation of surface pollution is washed into them in a comparatively few hours, but here again the majority of such pollution is of animal origin. India is for the most part the land of unpurified water-supplies, or to be more correct, a land where purification does exist, but it is brought about by purely natural agencies. If we desire to alter this state of affairs, or if any measures of improvement are to be started on scientific and sound business lines, it is absolutely necessary to be acquainted with the extent and power of these natural forces.

It must be obvious to even a mere casual observer, that in the tropics we have opportunities for a careful study of such problems as the fate of fæcal contamination added to a river or lake in purely natural surroundings. It is, of course, well known that for the most part intestinal organisms rapidly die out in water, but we shall see many live for months, others actually multiply, and the whole aspect of a pollution passes through certain well-defined stages. It has been explained that in England there is little opportunity for studying these problems, but something has been done. Houston's important research on storage of raw river waters must be mentioned. He has shown that a certain percentage of organisms which grow on gelatine at 20°C. disappears in a specified time, that a certain percentage of organisms which grow on agar at 37°C. disappears in the same time, that a certain percentage of bacilli which ferment glucose and lactose, and give the indol reaction, also die out after a certain period of storage. But this tells us absolutely nothing as to the bionomics of the process of self-purification. Such questions as—Is there any order of procedure in this action? Are there any indications which will tell us how far the process has gone at any given time? How far does the process go on in natural surroundings, or is it confined to storage reservoirs? are left unanswered. Now we shall show that in India we practically never have a water like the raw Thames water used by Houston. Most of our natural sources would be more comparable with his samples taken half way through, or approaching the end of, the period of storage, consequently many of our raw waters do not contain any " typical coli " (as defined by him), and yet they are loaded with bacteria derived from fæces. Hence it is obviously necessary to study the purifying process in greater detail—we must see the inner working of it—if we want to make full and economic use of it. It must be understood that it is not our intention to underestimate the value of the conclusions that can be drawn from Houston's work; to the water engineer, whose duty it is to convert a dilute sewage into a drinking-water, they are of fundamental importance, and their practical application is too obvious to admit of doubt. All we wish to say is that we in India frequently start where he leaves off, so we must pursue the study further.

The study of the self-purification of surface water will, however, be looked at from the point of view of a water analyst rather than that of a biologist. The questions that such an officer has to ask himself are—(1) Is there any evidence of fæcal pollution? (2) Is there anything in the results obtained which gives any idea as to when the pollution was added to the water? and (3) What is likely to be the result in a community if the water is used for drinking purposes?

In unfiltered supplies in India the answer to the first of these is always in the affirmative, except in the case of large rivers in the height of the dry season. Fæcal pollution is always present in water to a greater or less extent; consequently, the necessity for being able to answer the last two questions is really great, as energetic action in order to save life may hang on the decision.

In investigating any sample of water, two different methods are available: (1) The analyst may try to find in the sample the actual pathogenic organisms themselves, such as cholera vibrios, bacillus of enteric fever or members of the group of organisms of the para-typhoid and Gærtner class. (2) From a study of the general flora of the sample as a whole he may seek for evidence of recent fæcal pollution, assuming that this kind of contamination is always potentially dangerous. Both of these methods have their uses, and both are to be found in every-day operation in laboratories. The first method is made use of in routine practice when one suspects that the water is the cause of the spread of cholera. The technique is both simple and reasonably accurate, so that a negative result may be looked upon as fairly satisfactory evidence that vibrios are really absent. This remark, however, cannot be said to apply to the other pathogenic intestinal organisms. It is true that Starkey of McGill University has proposed a method of separating out and identifying certain members of the above groups, and although admitting the value of his method when a positive result is obtained, a negative result is less satisfactory. Again, the difficulty in isolating Eberth bacillus from natural water is too well known to require anything more than a passing reference. Consequently, for ordinary work we have to rely for our answer to the two queries, on a study of the natural phenomena of the disappearance of fæcal organism from water.

Many analysts in England would probably take up the position that there are no thoroughly established facts which would justify any conclusion on such a point as whether a specified pollution was of recent date, or of old standing. They would state that whenever fæcal organisms are present in more than a certain number, that water was dangerous for human consumption. Some might go on to say that they considered organisms that gave certain fermentative reactions more objectionable than others which did not. As regards England, they are probably correct in making these assertions, but we shall show that, in the first place, this attitude would condemn nearly every drop of drinking-water in India, and in the second place, that, thanks to the natural conditions of the country, we have got a step further than this.

In practically every laboratory in Europe the presence of "true coli" is accepted as proof of undesirable contamination. This coli standard requires some discussion. It is stated by Houston that a class of organisms which give certain fermentative reactions are indicative of objectionable fæcal pollution. The reactions used are few, and quite a large number of distinct species of intestinal organisms fulfil these conditions. We shall show further on that all members of this group are *not* equally indicative of undesirable pollution. Thus, supposing *A* and *B* two separate organisms in this group, *A* may be found in nature to multiply in water, or to remain at the bottom of lakes for long periods. On the other hand, *B* may be clearly shown to disappear in a few days. Obviously, therefore, the significance of these two organisms would be different when looked at from the point of view of their presence in any sample of water. The point it is necessary to emphasize is, that a careful study of the natural phenomena of self-purification of water will tell us something of value concerning the life-history of individual species of fæcal organisms ; classifying organisms into artificial classes according to their action on certain sugars does not tell us anything. It appears to us that there is a tendency among water analysts generally to stick too closely to certain standards, useful though they may be, rather than to go to Nature herself for our information. It has already been stated that the arbitrary rules which are admittedly fairly satisfactory in England, would be worse than useless in India. Of course, classification of organisms according to their fermentative reaction is necessary in order to describe them. Thus, we shall use the term "lactose fermenters," "glucose fermenters" and also a group of organisms which are "glucose +, lactose −," but we wish it to be understood clearly from the very outset, that we never for a moment suppose that all organisms which ferment the same sugar, possess similar characteristics in other respects, or behave similarly when passed into water, and subjected to natural forces. In fact, we shall show clearly that this is not the case.

If any classification of organisms is necessary, a much more satisfactory method, from the point of view of water analysts, is to divide them according to their ability to live in water exposed to sunlight. Class I containing the susceptible organisms which rapidly die out on exposure to sun's rays : Class III the resistant bacilli, or those which will remain weeks, or months, in water : Class II the intermediate species, about which less is known. The fact that one variety of fæcal organisms will live 6 months at the bottom of a lake is worth something to an analyst, but of what use is the mere fact that such and such an organism liquefies gelatine ?

This briefly foreshadows the line of thought in this work ; we wish to study nature, not to attempt to fit nature into our laboratory ideas. If any facts in the life-history of fæcal organisms can be established, we are at liberty to apply these to our work on water bacteriology.

Something must be said on the subject of the method of analysis used in this investigation. The method which we shall make use of in this work is that suggested by Dr. A. MacConkey. It is not very different from that used in all other laboratories where bacteriological examination of water is carried out. A detailed description of the process will be found in appendix A.

CHAPTER II.

A STUDY OF THE BACTERIOLOGY OF FÆCES OF MEN AND OTHER ANIMALS.

As the object of this small work is to show the changes which occur after fæcal material has been added to water, it is obvious that a careful study of the bacteria found in fresh fæces is very important. The subject has been studied carefully for the last 4 years, the fæces of men and cattle being selected for the work. The reasons for the choice are many. First, because MacConkey has already partially investigated both these in Europe, and secondly, cattle are extremely numerous in this country, so that although pollution derived from towns is probably of a mixed origin, that derived from the surface washings of agricultural lands is derived almost solely from cattle. The subject will be divided into 3 separate headings :—

(1) A study of the lactose fermenters.
(2) A study of the class of organisms that ferment glucose but not lactose ; and
(3) A study of the fæcal streptococci.

Lactose fermenting organisms in fæces.

The various points which we wish to elucidate in a study of this subject are, (1) What organisms are common in fæces, and consequently may be expected to be present in polluted water ; (2) whether the results of the work by MacConkey and others on fæces in England are similar to those obtained in India ? (3) Whether any particular organism exists in human fæces that is not met with in cowdung or *vice versa ?* (4) Whether the relative percentage of various organisms, or the percentage in MacConkey's 4 groups, in human and animal fæces can be worked out so as to demonstrate the existence of any constant relation between the bacteriological flora in each. The work of MacConkey (Journal of Hygiene—19 July 1905 and 1906) shows that the relative percentages in the four groups differ in the dejecta of men and cattle, and that it may be possible to use these figures as a guide in ascertaining which of these two sources gives rise to any specified pollution. If we can give definite answers to these questions, we shall be in a position to compare the flora of fæces with that of water and to draw conclusions accordingly. Since July 1908 the subject of the flora of fæces has been under continuous investigation. Very many analyses have been carried out. The subject of the comparison between MacConkey's work and ours, and the use of his four classes will be first dealt with. The method adopted in these analyses is as follows :—

An emulsion is made with a fair quantity of fæces in water (usually one gram. in 9 c.c.). This emulsion may be treated in two ways : (1) A drop is placed in the centre of a bile salt neutral red lactose agar plate. This is wiped over the surface of the plate. The same glass wiper is then applied to two similar plates. The isolated colonies obtained are picked off after 18 to 24 hours' incubation at 37°C or 42 C and put through various tests.

(2) A few drops of the mixture are placed in bile salt lactose broth, and incubated for 18 hours. A loopful of the culture is then spread on the bile salt plates and the colonies treated in the same way. It may be stated that these methods give practically identical results. From a great deal of experience of both we find that the use of the broth culture does not alter the relation of organism in fæces. This is a rather important finding.

It should be remembered that it was suggested by MacConkey to divide all lactose fermenting organism into 4 groups, according as they did or did not ferment saccharose and dulcit.

Group (1) ferment neither saccharose nor dulcit ;
Group (2) ferment dulcit but not saccharose ;
Group (3) ferment dulcit and saccharose ; and
Group (4) ferment saccharose but not dulcit.

The figures are given below and the results obtained have been tabulated in Tables II (*a*) and (*b*).

TABLE II(*a*).

Human Fæces. (*Percentages*), 1,207 *organisms.*

	Series I.	Series II.	Series III.	Series IV.	Series VII.	Series VIII.	Series IX.	Series X.	Series XI.	Grand average.
Group I ..	46·6	54·0	44·0	52·0	78·0	52·2	37·4	70·0	40·9	53·2
Group II ..	25·3	35·0	40·0	12·0	4·0	1·1	14·1	·5	27·9	17·4
Group III ..	11·3	2·4	14·0	8·0	2·0	8·8	7·6	1·0	6·3	6·8
Group IV ..	16·6	8·9	2·0	28·0	16·0	37·7	41·0	27·5	24·4	22·2

TABLE II(*b*).

Cow Dung. (*Percentages*), 1,029 *organisms.*

	Series I.	Series II.	Series III.	Series IV.	Series V.	Series VI.	Series VII.	Series VIII.	Series IX.	Series X.	Grand average.
Group I.	35·3	58·3	52·0	36·0	40·8	36·8	42·7	52·2	19·3	29·9	40·3
Group II.	8·0	13·6	18·0	17·0	3·7	13·1	3·6	1·5	8·7	7·0	9·4
Group III.	22·0	6·8	16·0	10·0	22·2	9·4	5·4	5·9	36·7	16·0	16·0
Group IV.	34·6	21·7	14·0	37·0	33·3	40·6	49·1	40·3	35·0	46·8	35·8

MacConkey in his work on the bacteriology of fæces (1905) gives the following percentages among 241 lactose fermenting organisms isolated from human fæces :—

	MacConkey's figures. Per cent.	Our results 1,207 colonies. Per cent.
Group No. 1 ..	.. 34	53·2
Group No. 2 ..	.. 38	17·4
Group No. 3 ..	.. 15	6·8
Group No. 4 ..	.. 12	22·2

He goes on to say " only 4 organisms out of the 241 were shown definitely to belong to the bacillus lactis ærogenes type. Bacillus cloacæ was not isolated in one instance. The cane sugar fermenting bacilli numbered 65, while non-cane sugar fermenting bacilli reached a total of 176 " (about 1 to 3).

Figures obtained from our results are placed beside those given by MacConkey to facilitate comparison. Note that the difference is not very marked. Lactis ærogenes is not a common organism in human fæces in this country, but it is met with in 6 of the series ; it is entirely absent from 3 only. Cloacæ has been found occasionally (*vide* Table II*c*).

The proportion of saccharose and non-saccharose fermenters is 348 to 852 (about 1 : 2½). This increase in the non-saccharose fermenters in India, coupled with the fact that coli communis is a rare organism in fæces, emphasizes the difference between the two parts of the world, when considered from the standpoint of the water analyst.

It should be mentioned here, that the samples of human fæces used by us, were taken from the native population, who are almost entirely vegetarians ; it would not appear that the diet had any very great influence on the bacteriological contents.

In cowdung MacConkey (1905) gives the following percentage in his groups :—

	MacConkey. Per cent.	Ours. 1,029 colonies. Per cent.
Group No. I ..	16·6	40·3
Group No. II ..	25·0	9·4
Group No. III ..	47·9	16·0
Group No. IV ...	12·5	35·3

He also states " none of the organisms isolated of group 4 were identified as bacillus lactis ærogenes type." These percentages appear to be worked out on 48 colonies from six samples of cowdung ; so the data are rather small; it will be observed that the relation between the four groups in this country is very different from that in Europe. Lactis ærogenes is usually met with in small numbers in cowdung in this country. Bacillus cloacæ is sometimes extraordinarily common.

The proportion of saccharose fermenters to non-saccharose fermenters is 524 to 505 (about 1 to 1).

	Human. Per cent.	Cowdung. Per cent.
Group I	53·2	40·3
Group II	17·4	9·4
Group III	6·8	16·0
Group IV	22·2	35·3

Placing the averages for cowdung and human fæces side by side, it would appear that in human fæces group No. I predominated, in cowdung Nos. I and IV.

This conclusion may be interesting, but we doubt if it is of very much practical use to the water analyst. It would certainly not be safe to conclude, because the organisms in any sample of water (especially after exposure to the sun) followed the usual percentage of cowdung, or human fæces, that therefore cowdung, or human fæces, was the polluting agent.

MacConkey (1906) considers that in the examination of milk these figures may be of considerable value, but even this is doubtful.

In studying the figures (*vide* Tables II (*a*) and (*b*)) of the various series, it is apparent that there are very considerable differences in the percentage proportions at various times of the year. Compare, for instance, the percentages of series Nos. 1 and 7 in human fæces; again, series No. 4 human fæces might easily be mistaken for a cowdung average; it very closely resembles series No. 2 cowdung. Series No. 3 cowdung might easily pass for human fæces. Further, by reading the figures in the various groups, in Tables II (*a*) and (*b*) from left to right very considerable variations will be observed; apparently groups 2 and 3 are subject to the greatest irregularities.

The dividing of fæcal organisms into these classes is of no practical value. In the first place, it is a purely laboratory classification, and, in the second place, we shall show conclu-

TABLE II (*c*).

ES III.	SERIES IV.		SERIES. V.	SERIES. VI.	SERIES VII.		SERIES VIII.		SERIES IX.		SERIES X.		SERIES XI.
Cow.	Human.	Cow.	Cow.	Cow.	Human.	Cow.	Human.	Cow.	Human.	Cow.	Human.	Cow.	Human.
2·0	4·0	..	20·3	2·0	15·0	4·5	10·0	1·5	3·8	3·5	3·5	3·8	4·8
..	..	..	..	..	..	..	..	..	..	..	..	..	..
..	..	..	..	..	1·0	..	..	..	..	..	..	...	..
44·0	48·0	36·0	20·3	35·0	62·0	38·0	42·2	50·7	33·8	15·8	66·5	26·1	36·1
2·0	..	..	..	..	..	..	..	..	..	..	..	..	..
4·0	..	..	..	..	..	..	..	..	..	..	..	..	..
10·0	12·0	16·0	3·7	12·5	4·0	3·5	..	1·5	14·1	8·7	·5	7·0	27·2
..	..	..	..	..	..	..	1·1	..	..	..	..	..	..
..	..	..	..	..	..	..	..	..	..	..	..	..	·7
..	..	..	..	·6	..	..	..	..	..	..	..	..	..
8·0	..	1·0	..	..	..	..	..	..	..	..	..	..	..
..	..	1·0	1·9	..	..	..	..	..	1·3	..	..	..	..
..	..	..	..	..	..	3·5	7·7	..	..	..	..	·7	·7
4·0	..	..	..	..	..	..	..	..	..	3·5	..	15·3	..
8·0	7·0	9·0	20·3	9·0	2·0	1·0	1·1	5·9	6·4	31·5	1·0	..	5·6
4·0	1·0	..	..	..	..	..	..	..	..	1·7	..	..	..
..	..	..	..	..	..	3·5	..	..	..	..	..	..	..
2·0	1·0	..	1·9	..	..	7·0	20·0*	..	..	..	..	5·3	·4
10·0	27·0	29·0	31·5	40·0	15·0	32·0	17·7	38·8	39·7	28·0	27·5	41·5	24·0
2·0	..	8·0	..	..	1·0	5·5	..	1·5	..	..	..	..	..
..	..	..	..	..	..	..	..	..	..	..	..	..	..
..	..	..	..	..	..	..	..	..	..	7·0	..	..	..
50	100	100	54	160	100	100	90	67	78	57	200	130	268
3	9	9	4	16	10	11	9	7	8	6	16	10	28
	Dec. 1908	Nov. 1908		Jan. 1909	Feb. & Mar. 1909		April to June 1910		July to Oct. 1910		Nov. 1910 to April 1911		July to Sep. 1911

* All came from one sample of fæces.

sively that the bacteriological flora of fæces is subject to frequent changes at different times of the year. We now proceed to a study of the actual bacilli isolated. Table No. II (*c*) gives the result in percentages.

It will be seen that Table II (*c*) goes into much greater detail, and gives the percentage prevalence of separate organisms in the various series. A large number of very interesting points are demonstrated by this table. The most striking feature is the continual and steady presence of the Grunthal group, the coscoroba group, and, to a less extent, the coli group. Wide variations in percentage occur, however, and even entire absence of these bacilli is recorded in some series, but these organisms undoubtedly form the bulk of those present in dejecta of man and cattle. The main interest of this study, however, lies in the presence or absence of the rarer bacilli ; in this connection much has yet to be discovered.

Great variations, both in number and kind, occur in the flora of fæces at different times of the year. Compare, for instance, the results of series Nos. 1 and 9 taken in July 1908 and March 1909 ; note the very rich mixture in the human fæces in No. 1 and the few varieties in No. 7. Observe the relative number of coli communis in the two series ; also the number of cloacæ, and the number of coscoroba. The changes noticed to some extent apply to both human fæces and cowdung. Both human fæces and cowdung show a considerable falling-off in coli communis and in cloacæ ; on the other hand, both show a very great increase in such organisms as coscoroba. Another most striking example of the rapid change of the flora of the intestine of man and animal is to be found in series No. 3. This change is not so much in quantity as in quality.

We wish to lay stress on the fact that experience has shown that there are " epidemics " of certain organisms in the fæces both of man and animals. For instance, in the dry weather of 1908 in Madras bacillus cloacæ were extremely common, especially in the dejecta of cattle. Later on, in December of the same year coscoroba became extraordinarily prevalent. Quite recently, in the monsoon of 1911, coli communis predominated in human fæces to a very great extent, probably as much as 30 to 40 per cent. of all organisms present belonged to this variety, yet the organism is quite rare in the dry weather. For some entirely unknown reason these organisms which up to this time were exceedingly rare suddenly became very common.

It is true, as demonstrated by the Table II (*c*), that human fæces are apparently subject to much less violent variation than cowdung, but it may be fairly safely stated that whenever a prevalence of any organism is observed in one it is also to be found in increased quantities in the other.

We consider, speaking broadly, that Table II (*c*) bears out this. The falling-off in the number of coli communis in both cattle and human fæces in 1908 has been already pointed out. This fact has been noticed in all subsequent hot seasons. The mere figures do not represent how great is the change. We have sought through dozens of samples of human fæces and cowdung, in order to find one specimen rich in coli communis without success. In July and August 1908, 1909 and 1910 during monsoon weather about one sample in four was crowded with these organisms.

Note the presence in large numbers of cloacæ in cowdung in series Nos. 1 and 2, the smaller numbers in human fæces, followed by their absence from series Nos. 3, 4, 8, 9, 10 and 11 in human fæces, their absence from cowdung in series Nos. 5, 6, 9 and 10 and the reduced numbers in Nos. 2, 3, 4, 7 and 8.

Observe the steady increase in the numbers of coscoroba in both human fæces and cowdung.

The presence of organism No. 67 in fair quantity in series 1 in both human fæces and cowdung, and its disappearance from nearly all other series, is also significant.

We do not wish to push this too far because it cannot be said to be an absolute rule ; but from experience in the laboratory we are prepared to state that—

(1) there appear to be seasons when certain organisms are commoner than at other times ; different varieties of organisms are few in the hot weather, plentiful in the rains ;

(2) when an organism is found commonly in cowdung, it is nearly always present, though not necessarily in the same quantity in human fæces ; and

(3) it is usual to find the same organism in the water-supplies at the time.

TABLE II(*d*).

Table showing the percentage frequency of various organisms in Madras water-supplies in three quarters, 1908-09.

Organism.	River.			Lakes and reservoirs.		
	Quarter ending 30-9-08.	Quarter ending 31-12-08.	Quarter ending 1-3-09.	Quarter ending 30-9-08.	Quarter ending 31-12-08.	Quarter ending 1-3-09.
	%	%	%	%	%	%
B. coli communis	4·2	8·75	1	3·7	3·1	*Nil.*
Lactis ærogenes	15·3	5·0	15	3·7	26·8	12·3
Coscoroba ..	7·0	5·0	19	5·8	15·0	13·1
Cloacæ ..	18·0	8·75	5	43·8	13·1	7·7

In support of this latter statement we wish to draw attention to Table II (*d*) where it will be observed for the quarter July, August and September, bacillus cloacæ was fairly common in river water, very common in lakes. Analysis of human fæces and cowdung during the months of July and August also shows a marked prevalence of this organism. Similarly in the second quarter, in all the water-supplies, there was a great falling-off in the number of cloacæ, accompanied by a decline in its numbers in cowdung and fæces. Another striking example is the rapid increase in the prevalence of coscoroba in both human fæces, cowdung and the water samples contemporaneously—*vide* Tables II (*c*) and (*d*).

The third and perhaps the most important example is furnished by October 1908. At this period by accident it was discovered that in fæces of several animals and in a large number of the water-supplies some rare organisms were observed to be quite common. Table II (*e*) shows the results

TABLE II (*e*).

Tabular Statement of Results of Analyses during October 1908.

Place where samples came from.	Date.	RECENT RAINFALL.	BACILLI PRESENT.						Remarks.
		Inches.	Saccharose.	Adonite.	Dulcit.	Inulin.	V. & P.	Motility.	
Vizagapatam town supply	8th Oct. ..	*Nil*	..	..	..	..	..	..	No rare bacilli.
Madura town supply ..	13th ,, ..	4·5	+	–	–	+	–	–	No. 39.
			–	–	–	+	–	–	No. 10.
Trichinopoly town supply	20th ,, ..	3·0	+	+	+	+	+	–	Oxytocus perniciosus.
			+	+	–	+	+	–	No. 69.
Tanjore town supply ..	21st ,, ..	3·0	–	+	–	+	–	–	No. 39.
			+	–	–	+	–	–	No. 110.
Well in Central Jail, Tanjore	21st ,, ..	3·0	+	+	+	+	+	–	Oxytocus perniciosus.
			+	+	–	+	+	–	No. 69.
Adoni town supply ..	27th ,, ..	*Nil*	..	..	..	..	..	..	No bacilli.
Cuddapah town supply ..	28th ,, ..	12·5	+	+	+	+	+	–	Oxytocus perniciosus.
King Institute well, Guindy, Madras ..	24th ,, ..	15·5	+	+	+	+	+	–	Do.
Red Hillslake, Madras	25th ,, ..	14·3	+	+	+	+	+	–	Oxytocus perniciosus.
	31st ,, ..	24·6	+	+	–	+	+	–	No. 69.
			+	+	–	+	+	–	Do.
Human fæces, Madras ..	End of Oct.—Early Nov.	33·0	–	+	–	+	–	–	No. 39.
			–	–	–	+	–	–	No. 10.
			+	+	–	+	–	–	No. 67.
Cowdung, Madras ..	End of Oct.—Early Nov.	33·0	–	–	–	+	–	–	No. 39.
			–	–	–	+	–	–	No. 10.
			+	–	–	+	–	–	No. 67.
Goat fæces, Madras ..	26th Oct.	17·0	+	–	–	+	–	–	No. 110.
			–	+	–	+	–	–	No. 39.
			–	–	–	+	–	–	No. 10.
			+	+	–	+	–	–	No. 67.

given in that particular year and the following points should be observed :—

(1) That the presence of these organisms is apparently contemporaneous with very heavy rain or monsoon conditions.

(2) That the places where samples are taken are far apart; in other words, the prevalence of the organism is observed over very wide areas.

(3) That they all disappear with great rapidity from both fæces or water-supplies. Since the year 1908 we have carefully looked out for these rare organisms, and we may point out that the same conditions have been observed in Madras, Bengal, and as far north as Allahabad, at exactly the same time of the year. The same group of organisms seemed to appear at certain times, always preceded by very heavy downpours of rain, and they disappear when the heavy monsoon is over.

In order to explain these parallel variations we have come face to face with the question—Is it the flora of the fæces which determines the organisms in the water, or is the reverse true ? And have the organisms present in the water any influence on the contents of the fæces ? There can be little doubt that under some circumstances the first of these questions may be answered in the affirmative. Although we are at present entirely ignorant of the cause of the appearance of the rare organisms in the discharges of man and animals in the month of October, there can be no reasonable doubt that the monsoon conditions, existing at the time, washed them into the water-supplies. This may also explain the coscoroba prevalence in December, January and February, for rains had been more or less common in November, December and the first week in January. But it certainly cannot explain the relation between the prevalence of cloacæ in both water and fæces in August ; for first, during the months of July and August, there was practically no rain ; and, secondly, we know what natural influences caused cloacæ to be so numerous in the water at this time, *viz.*, that the organism is one of the last to survive in a stored water.

Therefore, as it is certain that the fæces did not directly contaminate the various water-supplies at this time, it is all the more remarkable that the prevailing bacteria present in water and fæces should be identical, and it suggests that a reverse action is taking place, namely, that the organisms prevalent in the water-supplies are influencing the bacteriological contents of the intestines. At one time in the course of our investigations we were somewhat inclined to this view. The experiment given by MacConkey (Journal of Hygiene, 1905), where he inoculated the food of a cat with an outside organism, and recovered it in the fæces 24 hours afterwards, seemed to support this theory. In order to test the truth of this surmise, certain experiments were made in the King Institute, to see if it were possible to demonstrate any relation between the organisms present in an artificially contaminated drinking-water supply, and the fæces of animals drinking such water. Altogether about eight calves were selected. They were given King Institute well water morning and evening; but to each bucket a large number of one of the following organisms were added—oxytocus perniciosus, organism No. 97, cloacæ, lactis ærogenes and coli communis. At periods varying from one day to a week (the feeding experiments being kept on all this time), the fæces were examined; in no case were we ever able to recover in the fæces of the calves the organism that was present in the drinking-water. Great care was taken not to alter the daily routine of the animal's life; no drinking-supply, other than the artificially contaminated water, was allowed. Very large quantities of the organisms were given. It is true the bacilli used were derived from laboratory cultures, but this would not be likely to vitiate the results. The negative results obtained in these experiments would appear to show that the organisms taken in drinking-water produce no alteration in the intestinal flora.

There is also a further point which may be mentioned. Throughout the months of October, November and December, lactis ærogenes was an extraordinarily common organism in all the water-supplies. Yet there was no correspondingly increased prevalence of this organism in the samples of fæces examined at that time of year. Similarly in March and April 1910, a glucose fermenting organism was by far the commonest organism in Calcutta drinking water, yet there was no increase in the number of the species in fæces. Consequently, one is forced to the conclusion that the presence contemporaneously of bacillus cloacæ in both fæces and water-supplies is not yet satisfactorily explained. It will be noticed that we are still without any trace of explanation of the conditions which bring about these changes in the bacteriological contents of the intestinal canal of man and animals, and after four years' work we are not prepared to make any suggestions. That such influences exist, and that they are active over a very wide area, is certain.

In the light of these remarks nothing more need be said on the utility of attempting to establish an approximate percentage composition of the intestinal bacilli in the dejecta of different animals. Even the percentages in the four large groups, described by MacConkey, are of so little value that only the vaguest generalizations are possible.

We have never found any fæcal organism special to the intestinal tract of either man, cows or goats. It is true that a very few rare organisms found in cowdung have not yet been isolated from human fæces, but it is probably only a question of time until they are found to be present there also. On the other hand, we have not isolated a single organism from human fæces, that has not been found in cowdung at one time or another. For some time we thought organism No. 33 was confined to the intestinal tract of man, but it was eventually obtained from cowdung. Not only is it true that " at present there is no means of differentiating the lactose fermenting organisms of human from those of animal origin " (MacConkey 1905) ; but there is probably not a single lactose fermenting organism that is peculiar to any given animal.

More work is required on this very interesting subject of bacteria in fæces ; at present we can only be said to have pointed out a few leading lines, and to have discovered certain problems, of the explanation of which we are at this stage entirely ignorant. We consider the following wide conclusions are justifiable :—

(1) That the flora of the intestinal tracts of men and animals are subject to very considerable changes, due to influ-

ences which are at present unknown. These influences have been proved to operate over very wide areas.

(2) That within certain very wide limits, these forces appear to affect man and animals equally, both as regards number, and kind of micro-organisms.

(3) That under well defined conditions, such as heavy rainfall, the water-supplies contain the same organisms as the fæces of men and animals at that particular time; but that this similarity of bacteriological flora is also noticed occasionally when rain is absent and there is no apparent cause for it. The explanation of this occurrence is at present unknown.

(4) That having regard to the variation in the bacteria in fæces, both in quantity and kind, no constant approximate composition can be arrived at. Even in the large groups, suggested by MacConkey, variation in percentage composition in the same animal is considerable.

(5) No lactose fermenting organism has been isolated by us that has been proved to be the inhabitant of the intestinal tract of cattle, or man only.

(6) That the numerical relation of the organisms constituting MacConkey's groups in the intestine of cattle in India is entirely different from that in England; while in the intestines of man it appears to be very similar in the two countries.

(7) That a study of the organisms present in fæces at different times of the year is necessary for the proper interpretation of the results obtained from water analyses.

CHAPTER III.

BACTERIOLOGY OF FÆCES (*continued*).

STUDY OF A GROUP OF BACILLI THAT FERMENT GLUCOSE BUT NOT LACTOSE.

IN our researches into the bacteriology of water it has been discovered, as will be shewn later, that a class of organisms which ferment glucose but do not ferment lactose is not only exceedingly common in water, under certain climatic conditions, but the significance of these bacteria is of great importance to the water analyst; hence it has become necessary to study them in fresh fæces. In order to do this a careful research was carried out on exactly the same lines as that already described in Chapter II. The experiments will be divided into the following series :—

Series I.—8 samples of cowdung were taken and an emulsion of each was made. A small quantity of each was placed in the centre of a bile salt neutral red *glucose* agar plate. From 7 plates 20 colonies were picked off and from 1 plate 12 colonies (making a total of 152 in all); these were inoculated into the usual glucose and lactose fermentation tubes. Out of these 152 colonies 150 fermented lactose and glucose; 2 fermented glucose only, none fermented neither lactose nor glucose; in other words 98·6% of all the colonies fermented both lactose and glucose and 1·4% fermented glucose but not lactose.

Series II.—A precisely similar experiment was carried out with 14 samples of human fæces, 272 colonies from these samples being tested with glucose and lactose tubes. Out of these 272 colonies, 255 or 93·7% fermented both lactose and glucose; 17 or 6·3% fermented glucose but not lactose. Only 5 out of the 14 samples contained any of this latter class.

Series III.—A small portion of 12 separate samples of human fæces were inoculated into separate tubes of bile salt neutral red glucose broth. From these tubes, isolated colonies were obtained by planting out on bile salt glucose agar. 144 colonies from these 12 samples were tested as to their power of fermenting glucose and lactose. Out of these 144 colonies, 138 or 95·5% fermented lactose and glucose; 6 or 4·5% fermented glucose but not lactose.

Series IV.—A precisely similar experiment was carried out with 12 samples of cowdung. Out of 144 colonies treated in this way, 135 or 93·8% fermented both glucose and lactose; 9 or 6·2% fermented glucose but not lactose.

In no case in any of these series (derived from 46 different samples of fæces and no less than 710 colonies) did a single organism that fermented lactose not ferment glucose, and no specimen of bacillus that fermented neither lactose nor glucose was obtained.

These results obtained from fæces are sufficient to show that by far the most important organism in fæces is a class of organisms that ferment both lactose and glucose; that in both human fæces and cowdung the class lactose – glucose + is distinctly rare and did not form more than about 5% of the intestinal flora at this time of year. It may be stated that these experiments were carried out in 1909. As we have shown previously in all work on fæces it is extremely dangerous to say that any particular variety of organism is always present in fæces in any constant percentage; it was very desirable to confirm the previous results by further investigations. In the months of February and March 1911, a further series of colonies were collected. Some of these were put through glucose broth, and plated out on lactose solid medium, and some were plated out direct on to a lactose solid medium.

Series V consisting of 1,728 colonies from 10 different samples of human fæces showed only 69 that belong to the class of glucose + lactose. – This works out at about 4%, the remaining 96% being glucose + lactose +. A similar series (*Series VI*) was obtained from cowdung. Out of 80 colonies that were grown in glucose broth and separated out on a lactose medium, 4 belong to the class of glucose + lactose –. This is 5% of all colonies.

In *Series No. VII* human fæces were plated direct on to solid medium. Out of 707 colonies from human fæces on lactose solid medium none belonged to the class glucose + lactose –.

All these previous results have been obtained on bile salt neutral red lactose agar. In order to check the accuracy of these it was thought desirable to make a few estimations with medium of another nature. Consequently Conradi Drigalski medium was tried.

Series VIII.—9 separate samples of human fæces were wiped direct on to these plates. The total number of colonies on all plates was not estimated, but whenever a plate containing discrete colonies was obtained, every colony was put through glucose and lactose broth. In this way 159 colonies were obtained; out of these 141 or about 88% fermented glucose and lactose and 18 or 12% belong to the class glucose + lactose –. It should, however, be stated that 16 out of these 18 colonies all were obtained from *one specimen* of fæces. This was evidently one of those rare occasions when these particular organisms were unusually numerous in fæces.

Series IX.—Exactly the same work was carried out with 5 different samples of cowdung in which case 99 organisms fermented glucose and lactose and one was glucose + lactose –. Series Nos. VIII & IX were done in the autumn of 1911.

It would, therefore, appear that this class of organism glucose + lactose, – is rare in the fæces of human beings and animals; and that investigations carried out over a considerable number of years show that they seldom exceed 5 per cent. of all organisms that grow in bile salt and very frequently they are present in smaller percentage. The results obtained show that rarely, one sample in eighty-four, they may be rather numerous, that is to say, as much as 20% or 30% of the total coliform organisms. In the course of our experiments we have found several instances when no members of this class can be isolated from a particular specimen, even when very large numbers of colonies were investigated.

Some study has been made of the fermentative reactions of this group which give glucose + lactose –. We have used the same sugar reactions that we always use for lactose fermenters, *viz.*, Saccharose, Dulcit, Adonit, Inulin, Vosges Proskauer's reaction, Indol, Motility. Probably it would be better if mannite had been included amongst the list. We do not pretend to have completed the work on this particular group, but Table III (*a*) gives the results we obtain up to date. As in the case of the lactose fermenters they are divided into series giving the date when the work was actually carried out.

TABLE III (*a*).

Non-Lactose Fermenters. Percentages. Human Fæces & Cowdung.

	Sacchar.	Dulcit.	Adonit.	Inulin.	Vosges & P.	Indol.	Motility.	Series I.	Series II.	Series III.	Series IV.	Cowdung Series V.
1	–	–	–	–	–	+	– . .	31·2	78·6	65·2	10·0	6·2
2	–	–	–	–	–	+	+ . .	43·7	*Nil.*	*Nil.*	37·0	. .
3	–	–	–	–	–	–	– . .	*Nil.*	*Nil.*	*Nil.*	7·0	46·8
4	+	–	–	–	–	–	– . .	*Nil.*	14·3	8·7	1·0	3·1
5	+	–	–	–	–	+	– . .	*Nil.*	*Nil.*	*Nil.*	26·0	12·5
6	+	+	–	–	–	+	– . .	*Nil.*	*Nil.*	*Nil.*	3·0	12·5
7	+	+	–	–	–	+	+ . .	*Nil.*	*Nil.*	*Nil.*	1·0	. .
8	–	+	–	–	–	+	– . .	*Nil.*	*Nil.*	*Nil.*	8·0	6·2
9	–	+	–	–	–	+	+ . .	*Nil.*	*Nil.*	*Nil.*	1·0	. .
10	–	–	+	–	–	+	+ . .	18·7	*Nil.*	*Nil.*	2·0	. .
11	–	–	+	–	–	+	– . .	6·2	*Nil.*	26·1	1·0	. .
12‡	+	–	–	–	+	–	+ . .	*Nil.*	7·1	*Nil.*	*Nil.*	15·6
No. of organisms total . .								16	14	23	97	32
No. of samples analysed . .								3	5	7	40*	13†
Time of year								April 1910	Nov. & Dec. 1910	Feb. & March 1911	Aug. & Sep. 1911	Aug. & Sep. 1911

* Only 14 showed non-lactose fermenters and in 26 none could be got.

† Only four of these showed any white colonies.

‡ Bacillus P.

Considering the small amount of research we do not wish to draw conclusions from this. It is, however, necessary to point out one fact, namely, that, as far as one can ascertain, organism No. 12, subsequently called Bacillus P., is not particularly numerous amongst the members of this group.

The importance of this fact will be seen later on when we come to deal with the presence of these organisms in water.

This concludes what we have to say on this particular group of organisms. The points which we wish to lay particular stress on, and which will be of considerable importance later on are :—

(1) That, from investigations carried out at different times during four years, we find that the particular group of organism whose fermentative reactions are glucose + lactose –, are rare in the fæces of human beings and animals as a whole, 5 per cent. or under representing their numerical value in a mixture. The common fæcal organism ferments both lactose and glucose.

(2) As far as can be ascertained at present "epidemics" of these organisms have not been met with, during the three or four years that the study of fæces has been going on.

(3) Separating the organisms out with sugar reactions does not show that any member of the group is particularly common; it is to be observed that organism P. is rare rather than otherwise.

CHAPTER IV.

BACTERIOLOGY OF FÆCES—(*continued*).

STREPTOCOCCI IN FÆCES.

IT is well-known to all acquainted with the bacteriology of fæces that streptococci of various classes are extremely common in this material. The honour of investigating the subject belongs to Dr. Houston who, as early as 1898, published his work on the presence of these cocci both in water and in fæces. It is with his more recent work that our results will be compared, in order to show the differences that appear to exist between England and India. As pointed out by Dr. Houston, streptococci are extremely numerous in fæces in European countries. In fact, he says that they are, roughly speaking, about as numerous as the coliform organisms. By obtaining a hundred stabs from different masses of fæces obtained at a sewage works, he estimates that the number of organisms in a gramme of fæces works out about as follows :—

1 Streptococcus was isolated from 1-10,000,000th gramme of the fæcal mixture.

27 Streptococci were isolated from 1-1,000,000th gramme of the fæcal mixture.

72 Streptococci were isolated from 1-100,000th gramme of the fæcal mixture.

Table No. IV (*a*) gives the results of the enumeration of the number of streptococci in fæces in this country. The first series were carried out as follows :—

A gramme of fæces was mixed with 9 c.c. of tap water : and a series of dilutions was made ; these were made 1-10, 1-100, 1-1,000, etc. The presence of streptococci were sought for in 1 c.c. of these dilutions in the usual way, by wiping the mixture over Conradi Drigalski plates. The highest dilution that showed any small colonies which microscopically proved to be streptococci is given in this Table.

TABLE IV(*a*).

Results of investigation of Streptococci in fæces.

No.	Date.	Highest dilution streptococci isolated.	No. of streptococci on plate.	No. of coli form organism on plate.	Kinds of Streptococci.
	Human fæces.				
1	27-10-10	·00001 gram.	2	..	Lamisacsal.
2	4-11-10	·00001 ,,	14	..	Lamirasacsal.
3	21-11-10	·00001 ,,	2	5	Do.
4	4-12-10	·0001 ,,	51	6	Do.
5	2-3-11	·0001 ,,	15	141	Do. & lamnasac.
6	13-3-11	·0001 ,,	3	57	Lamnasac.
7	9-3-11	·001 ,,	..	..	Plate not satisfactory.
8	31-10-10	·00001 (?)	..	..	Do. do.
9	20-1-11	None present	..	..	Do. do.
10	25-3-11	Ditto.	..	..	Do. do.
From crude fresh sewage—(Human origin).					
11	25-9-10	·001 dilut	50	150	Lamirasacsal lamirasac larasacsal.
12	26-9-10	·0001 ,,	11	67	Do. do.
13	29-9-10	·001 ,,	40	46	Do. do.
14	10-10-10	·01 ,,	..	..	Lamirasac.
15	18-10-10	·0001 ,,	2	5	Lamirasacsal.
16	19-10-10	·0001 ,,	4	13	Do.
17	22-10-10	·001 ,,	55	73	Do.
Calf dung.					
18	27-10-10	·01 gram.	..	..	Lamirasacsal.
19	31-10-10	·1 ,, (?)	..	..	Do.
20	21-11-10	·001 ,,	1	53	Do.
21	7-12-10	·1 ,,	5	19	Do.
22	31-1-11	·1 ,,	..	..	Do.
23	2-3-11	·01 ,,	45	10	Do.
24	4-3-11	·1 ,,	36	52	Do.
25	9-3-11	Nil. in ·1	..	..	Do.
26	16-3-11	*Nil.*	..	..	Do.
27	20-3-11	Nil. in ·1	..	..	Do.
28	25-3-11	·1 gram.	3	9	Do.

Many other analyses were made, but the results are discarded for one reason or another.

Each of the foregoing was made with one sample of fæces only. In the second portion of the table a crude sewage prepared for experimental work on septic tanks was used in order to prepare the plates. The fæcal discharges of 10 individuals are mixed with 50 gallons of water ; the hard masses on this occasion were *not* carefully emulsified (for reasons which do not concern this work), so that this particular mixture cannot be looked upon as being of exactly the same strength from day to day (as a matter of fact, the 4 hours' oxygen figure shows it varies very little) ; 1 c.c. of this mixture was diluted into 10, 100, 1,000, and 10,000, and plates were made in the usual way.

In the table are also given the number of coliform colonies which grow upon Conradi Drigalski, side by side with the small streptococci colonies. This is an useful piece of information, because if there was a numerical disproportion between the two it would be apparent from these plates.

The third part of the table gives the results obtained from cowdung, the method of examination being the same as for human fæces.

The points which are apparent from this table, are first of all that streptococci are extremely numerous in crude fæces, frequently being present in 1-10,000 and 1-100,000 of a gramme. They do not appear to be frequently found in smaller quantities than this. Also it is apparent from this table that streptococci are, roughly speaking, about as numerous as lactose fermenters, though comparing these with Dr. Houston's figures both streptococci and coliform organisms seem to be much fewer in number per gramme. The number of any kind of organism per gramme of fæces is obviously an unsatisfactory method of counting, though it is difficult to suggest a better, because fæces does not necessarily contain a constant quantity of water all over the world. It is possible that the fæcal material in this country contains more water and less solid material than it does in England, but whatever is the reason we find that both coliform organisms and streptococci are considerably less numerous per gramme of crude fæces in the tropics, than they are in a cold climate.

In making these statements, however, we wish it to be noted that it is highly probable that the number of streptococci per gramme of fæces varies at different times of the year. Thus, we have obtained a certain amount of evidence to show that in the dry weather they appear to be fewer than in the rains.

Table IV(*b*).

Classification of Lactose Positive Streptococci.
Houston's and Indian results.

	Human Houston.	Human Houston.	Human Indian.	Cowdung Houston.	Cowdung Indian.
	1903-4.	1908.	1910-11.	1904-5.	1911.
Lamamisacsal	23·14	..	..	..	..
Lamisacsal	15·73	..	..	..	..
Lamirasacsal	15·28	44	92·1	58·82	74·3
Lasacsal	11·79	..	..	10·59	..
Lamisal	11·35	..	..	3·35	..
Larasacsal	6·99	..	..	17·65	25·7
Lamisac	6·11	..	·9	1·18	..
Lamamisal	3·49	..	..	..	..
Lamamirasacsal ..	2·18	4	..	..	..
Lamasacsal	1·75	..	..	..	..
Lamarasacsal	·87	..	..	..	..
Lamirasal	·44	49	1·7	..	..
Lamirasac	·44	1	2·6	1·18	..
Lamisacsal	..	..	..	3·53	..
Lasac	·44	..	2·6	1·18	..
Lamira	..	2	..	1·18	..
Larasal	..	..	..	1·18	..
	229 colonies	100 colonies	115 colonies	85 colonies	39 colonies

La = lactose +, ma = mannite +, mi = milk clot, ra = raffinose +, sac = saccharose +, sal = salicin +.

As regards the varieties of streptococci classified according to their fermentative reaction we have again to quote Dr. Houston's work. Table No. IV (*b*) gives the result of Houston's researches on human fæces and cowdung, and our own placed side by side. It will be observed that part of Houston's work on human fæces was carried out in 1903-04 and part in 1908. The great difference between these series of results should be carefully noted, because they themselves bear out our contention that there is constant variation in the flora of fæces generally.

From a comparison between the four columns it will be found that as far as these figures can be trusted, very great difference exists between the kinds of fæcal streptococci in the two countries. The Indian streptococci seem to be mostly of one class with very few exceptions. Still no less than 92 per cent. of the streptococci isolated from human fæces give the typical reactions mentioned by Houston. Consequently we may agree with his conclusion :—

" Inasmuch as salicin, as well as lactose, is really attacked by streptococci, it might be convenient to accept 'lactose salicin microbes' as a basis for classification purposes and of the streptococci fulfilling these preliminary characters to look with special disfavor on those which failed to attack mannite but clotted milk, and which also attacked both raffinose and saccharose (lamirasacsal)."

CHAPTER V.

THE PREVALENCE AND SIGNIFICANCE OF STREPTOCOCCI IN WATER.

THIS subject follows closely on what has gone before, and, as no very great amount of space will be required in disposing of it, it seems advisable to complete the study of streptococci and their significance in water supplies.

We have already shown that streptococci are extraordinarily common in fæces both of man and animals. Consequently it is obviously necessary to devote a certain amount of attention to an enquiry into what becomes of the millions of streptococci which undoubtedly pass into surface water along with fæces. In order to elucidate this problem two separate series of researches have been carried out. First of all our routine samples of water which come into our laboratory from various town supplies, rivers, lakes and wells, etc., have been carefully examined for the presence of these cocci. Secondly, experimental mixtures of fæces and water have been examined under varying conditions.

Before proceeding, it may perhaps be well to mention that the method of searching for streptococci in water does not differ materially from that already described in the foregoing chapter. For raw waters, we take 20 c.c. of the sample, centrifugalise it for a period of a quarter of an hour in a centrifuge, the sediment is carefully pipetted off, put into lactose broth, from this on to large Conradi Drigalski plates. The small colonies are picked off, examined under the microscope to see if there are true streptococci; they are then tested by putting them through the following reagents, —milk, mannite, lactose, saccharose, salicin and raffinose broth. The method is identical with that used by Houston except that for his raw water 1 c.c. appears to be usually sufficient.

From a very careful study of many samples from rivers, lakes, and wells we find that the number of occasions on which streptococci have been found is extremely small. With one or two exceptions they are practically confined to the samples taken from the Hooghly water in the neighbourhood of the town of Calcutta itself. Here, occasionally, streptococci of the usual fæcal varieties can be isolated; of course, the pollution in these samples is extremely great, as a considerable portion of Howrah and Calcutta is situated above the point of sampling. Besides this, the boat traffic on the Hooghly is considerable; the population living entirely on boats is estimated at about 30,000 souls. Even under these circumstances streptococci can only be obtained from large quantities of water, and on few occasions only. Once these cocci have been isolated from water in the intake at Howrah Water Works. Inspection shows that there is a good deal of pollution of the bank immediately above this. One well in a jail which showed rather gross pollution, also yielded a few streptococci.

Out of 378 samples, 151 of which were raw waters, 6 showed streptococci in 20 c.c.; in all these other evidence of gross pollution was obtained, 3 were taken from the Hooghly near Calcutta. It may, however, be stated that even in the height of the monsoon, when the large rivers contain a great deal of gross pollution from the land, streptococci cannot be found in 20 c.c. of the water.

Houston in his report gives a table of his results obtained from the raw Thames, Lee and the New River water for the year 1909 working with 1 c.c. of the water; he obtained the following results:—

"Thus 1908 (764 + 896 + 248) sub-cultures were made from 1 c.c. amounts of 156 (52 + 52 + 52) samples of raw river water (Thames, Lee, New River). 28 (13 + 13 + 2) of these samples contained streptococci and the total number of streptococci isolated was 71 (19 + 50 + 2)."

"It should be noted, however, that in three instances (Thames, two sample; Lee, one sample) the 1 c.c. plates were so crowded that it was necessary to make the subcultures from the 0·1 c.c. plates."

"These results confirm those of last year (1908) and show that the raw waters seldom contain any living fæcal streptococci in 1 c.c. of water and that even when present the number is usually small (71 streptococci out of 1908 subcultures).''

From these figures it will be seen that the results obtained in India and in Europe coincide to a remarkable degree; only the facts observed in England appear to be more intensified in this country. Streptococci are rare in such polluted rivers as the Thames and the Lee in England. They are infinitely rarer in rivers and lakes in India. On the other hand, they are occasionally met with in places where recent and gross pollution takes place.

The contrast between the results obtained from fresh fæces and our water-supplies is very great.

Obviously the next point is to investigate the reasons for this rapid disappearance of an organism which is so common in fæces. In order to do this several experiments were carried out.

(1) Ordinary Winchester quart bottles were taken and filled with Calcutta tap water, which is free from streptococci. A few grammes of our crude sewage used in the Chemical Laboratory were added to this. The mouth of the bottle was blocked up with cotton-wool, and the mixture was allowed to stand. An analysis of this fresh mixture showed that there were usually between 100 and 1000 lactose fermenting, coliform organisms per c.c. of the mixture, and between 10 and 100 streptococci in the same quantity. The bottles were allowed to stand in the laboratory at a temperature of 80°—85°F. The mixture was carefully shaken up, and examined for the presence of streptococci daily. Ten such experiments were carried out; one of which, however, contained no streptococci at all in the original mixture. Of the other nine the results were as follows:—The cocci were present after 24 hours in seven bottles, in two they had disappeared. In three a few chains could be found in 20 c.c. of the emulsion after 48 hours, in six they had disappeared entirely. In 72 hours no cocci could be found in any of the mixtures. These experiments are particularly interesting, because they differ very materially from work that has been published by

Houston. The following para. gives the results quoted from his 5th Research Report :—

"'Tests were also made to determine the number of weeks, during which these 100 streptococci sustained their life in Pasteur-filtered West Middlesex main tap water. About 100,000 of each of the 100 varieties of streptococci were added separately to 100 tubes, each containing 10 c.c. of the water sterilised by 'Pasteur-filtration' and 1 c.c. cultures made therefrom each week.

"The tubes were kept in a dark cupboard the temperature of which varied from 46 to 61 degrees Fahrenheit during the progress of the experiments.

The following is a summary of the results :—

3 per cent. died in 1 week.
11 ,, ,, 2 weeks.
32 ,, ,, 3 ,,
38 ,, ,, 4 ,,
8 ,, ,, 5 ,,
4 ,, ,, 6 ,,
2 ,, ,, 9 ,,
1 ,, still alive in 9 weeks.
1 tube became contaminated.

"In my 1903-04 report to the Local Government Board, previously referred to, it was shewn that although some of the fæcal streptococci of experiment died very speedily in sterile tap water and sterile salt solution, others persisted for a long time, and I was unable to associate satisfactorily this variability as regards vitality with differences in the biological characters of the streptococci.

"It must be admitted that these vitality results appear to 'clash' with the observed fact as regards the paucity of streptococci in raw river water.

Nevertheless, the behaviour of fæcal streptococci in sterile water is not to be taken as necessarily measuring the vitality of these micro-organisms when in competition with a multitude of other microbes in raw river water."

It would appear that the varieties of streptococci used (they appear to be laboratory cultures and pure) retain their vitality in water much longer than do those organisms encountered in this country. Our experiments were not made with laboratory cultures, but with mixtures of fæces and water which contained all the usual varieties of intestinal organisms, as well as streptococci. Consequently, these two results are not strictly comparable.

A second series of experiments was made on a much larger scale. In the compound of the laboratory there is a very large tank or pond which has a surface of about an acre and a half. This tank was carefully polluted with a mixture of cowdung, fæces and effluent made up in large quantities. This evil mixture was spread over the surface of the tank just at sunset. The mixture always contained streptococci in large numbers. Morning and evening of the subsequent day samples were taken from the surface and from the bottom of the tank. The results are given below in tabular form :—

Table V(*a*).

Experiment No. I—Tank Polluted, 15-12-10.

Surface sample 1 c.c. 10 A.M., 16th, a few chains.
,, ,, ,, 4 P.M., 16th ,, ,, ,,
,, ,, ,, 10 A.M., 17th, no streptococci.
Bottom samples 1c. morning and evening, 16th, present.
,, ,, 1 c.c. 10 A.M., 17th, present.
,, ,, 4 P.M., 17th, absent.

Experiment No. II—Tank Polluted, 3-4-11, 4 P.M.

Surface sample 1 c.c. 8 A.M., 4th, present.
Bottom ,, ,, ,, ,, present.
Surface ,, ,, 4 P.M., 4th, absent.
Bottom ,, ,, ,, ,, present.
Surface ,, ,, 8 A.M., 5th, absent.
Bottom ,, ,, ,, ,, present.
Bottom ,, ,, 8 A.M., 6th, absent and all subsequent analyses.

Experiment No. III—Tank Polluted, 1-5-11, 4 P.M.

Surface sample 1 c.c. 8 A.M., 2-5-11, absent.
Bottom ,, ,, ,, ,, present.
Surface ,, ,, 4 P.M., ,, absent.
Bottom ,, ,, ,, ,, present.

EXPERIMENT NO. III—Tank Polluted, 1-5-11, 4 P.M.—(*contd.*)

Surface	sample	1 c.c.	8 A.M.,	3-5-11,	absent.
Bottom	,,	,,	,,	,,	present.
Surface	,,	,,	,,	4-5-11,	absent.
Bottom	,,	,,	,,	,,	present.
Surface	,,	,,	,,	5-5-11,	absent.
Bottom	,,	,,	,,	,,	present.
Surface	,,	15 c.c.	,,	6-5-11,	absent.
Bottom	,,	,,	,,	,,	,,

From both these sets of experiments (1 and 2), it may be concluded that natural streptococci die out with great rapidity when they are placed in water. The experiments also show that they sink to the bottom, where they are evidently in very uncongenial surroundings, for they perish very quickly. A study of the varieties of the streptococci which have been recovered from water supplies has not been carried out in this country, for the simple reason that very few colonies have actually been obtained.

From the point of view of the water analyst the significance of the presence of streptococci in any given sample is extremely apparent. Streptococci are extraordinarily common in fæces and extraordinarily rare in water. They die out with very great rapidity in the latter. Apparently the sunlight has nothing whatever to do with the rate of disappearance, for the cocci perish just as fast in a Winchester quart bottle in the laboratory, as they do in rivers and lakes. The presence of undoubted fæcal varieties is proof of the recent and dangerous character of the pollution under investigation. In India it is of little use looking for streptococci in ordinary water supplies, so a negative result does not increase our knowledge of the sample very much. Further, in cases of gross and recent pollution, evidence of other kinds is usually very conclusive, but the finding of fæcal streptococci is confirmatory evidence of very considerable value. One example of the usefulness of the test may be given, *viz.*, during an investigation on the Calcutta water supplies some samples of filtered water showed strong evidence of pollution of a most undesirable and objectionable character; in one case streptococci also were isolated. In this instance a highly polluted subsoil water (which probably came direct from a leaky sewer in the neighbourhood) was gaining access to an underground filtered water reservoir.

The following conclusions are quoted from Dr. Houston's work :—

> "(1) That streptococci are superabundant in human fæces.
>
> (2) That fæcal streptococci are absent or non-discoverable in a relatively large volume of pure water.
>
> (3) That fæcal streptococci do not multiply in pure water.
>
> (4) That some fæcal streptococci are of feeble vitality, and the presence of such streptococci in a water, if they could be differentiated from their more robust companions, would seem to indicate pollution of recent and therefore specially dangerous sort."

These also describe our experience in this country, only the results of our work show that we should be justified in stating the case somewhat more forcibly.

CHAPTER VI.

THE PREVALENCE AND SIGNIFICANCE OF A CLASS OF ORGANISMS WHICH FERMENT GLUCOSE BUT NOT LACTOSE IN SURFACE WATERS.

WATER analysts have long recognised that a class of organisms which ferment glucose, but not lactose, is very common in water, so much so that it is still a debatable point whether a glucose or lactose bile salt broth is the best medium to use in endeavouring to obtain a rough estimate of fæcal organisms present in water. In the original paper by MacConkey and Hill a bile salt glucose broth is recommended. Subsequently, however, the former of these authorities has stated that a lactose broth is better suited for the purpose, and that a glucose broth was used so as not to exclude bacillus of enteric, and the Gaertner group. On the other hand, Dr. Houston is of opinion that a bile salt lactose broth is not sufficiently inclusive for the first step in isolating from water the large group of organisms which are probably fæcal in origin. Following MacConkey's advice our original work at the King Institute, Madras, 1907-08, was done with lactose broth, but subsequently it seemed very desirable to investigate the use of glucose medium as well. So in July, August and September 1909 all routine samples, taken from water supplies all over the Presidency of Madras, were put through both glucose and lactose broth by separate assistants. This piece of work gave some very remarkable results, and showed the necessity for investigating the matter more thoroughly. As regards the method followed in this preliminary investigation very little need be said. The usual dilutions of 20, 10, 5, 1, ·1, ·01 of the water were made; one set being put into glucose and the other into lactose broth. From the 20 cc. tube a loopful was taken out, mixed with water and put on to a bile salt neutral red lactose agar plate. From each sample a number of colonies were picked off at

TABLE VI (*a*).

Comparative results of Glucose and Lactose Broth on Madras Water Samples.

	Lactose Broth.								Glucose Broth.								Remarks.
	20 c.c.	10 c.c.	5 c.c.	1 c.c.	·1 c.c.	·01 c.c.	Lactose +	Glucose +	20 c.c.	10 c.c.	5 c.c.	1 c.c.	·1 c.c.	·01 c.c.	Lactose +	Glucose +	
1 Dodabetta Upper	+	+·	−·	−	−	−	10	10	+	+	+	+	+	−	10	12	
2 ,, Lower	+	+·	−·	−	−	−	10	10	+	+	+	+	−	−	9	12	
3 Tiger Hill	+	+	−·	−·	−	−	10	10	+	+	+	+	+	+·	0	12	
4 ,, ,, Pipe		*nil.*	*nil.*									*nil.*					
5 Dodabetta Pipe Line	+	−	−·	−·	−	−	10	10	+	+	+	−	−	−	0	12	
6 Marlimund	+	+	+	+	+	−·	10	10	+	+	+	+	+	+	0	12	
7 ,, Pipe Line	+	+	−	−	−	−	10	10	+	+	+	+	+	−	...	...	
8 Kodapamund	+	+	+	+	−	−	10	10	+	+	+	+	−	−	9	12	
9 Old Ootii	+	+	+·	−·	−	−	10	10	+	+	+	+	+	+	9	12	
10 Guntur Spring	+	+	+	−·	−	−	10	10	+	+	+	+	+	+	2	12	
11 ,, Filtered	+	+	+	+	−	−	10	10	+	+	+	+	+·	−	0	12	
12 ,, Tap	+	+	+	+	+	−	10	10	+	+	+	+	−	−	10	12	
13 ,, Tap	+	+	+	+	−	−	10	10	+	+	+	+	+	−	10	12	
14 ,, Tap	+	+	+	+	+·	−	10	10	+	+	+	+	+	+	9	12	
Madras																	
15 Sholavaram Tank	+	+	+	+	+·	+·	10	10	+	+	+	+	+	+	10	12	
16 ,, Channel	+	+	+	+	−·	−	10	10	+	+	+	+	+	+	6	12	
17 Red Hills	+	+	+	+	−·	−	10	10	+	+	+	+	+	+·	5	12	
18 ,, Tap	+	+	+·	−·	−	−	10	10	+	+	+	+	+	+	6	12	
19 ,, Tap	+	+	+	−·	−	−	10	10	+	+	+	+	+	+·	2	12	
20 Kurnool	+	+	+	+	+	+	10	10	+	+	+	+	+·	+·	9	12	
21 ,, Settling Tank	+	+	+	+	−·	−	10	10	+	+	+	+	+	+·	5	12	
22 ,, 2nd ,,	+	+	+	+	+·	−	10	10	+	+	+	+	+	+·	8	12	
23 ,, Filtered	+	+·	+	+	−	−	10	10	+	+	+	+	+·	−·	8	12	
24 ,, Tap	+	+	+	−	−	−	10	10	+	+	+·	+	+	+	1	12	
25 ,, Tap	+	+	+	−	−	−	10	10	+	+	+	+	−	−	7	12	
26 ,, Tap	+	+	−	−	−	−	10	10	+	+	+	+·	−	−	10	12	
27 Coonoor	+	+	+	−	−	−	10	10	+	+	+	+	+	−	4	10	2 Lactose—Glucose—
28 ,, Reservoir	+	+	+	+	−	−	10	10	+	+	+	+	+	−·	1	12	
29 ,, Tap	+	+	−·	+	−	−	10	10	+	+	+	+	−	−	12	12	
30 ,, Tap	+	+	+·	−·	−	−	10	10	+	+	+	+	+	−	12	12	
31 Adoni Spring	+	+	+	+	+·	−·	10	10	+	...	...	...	..	...	9	12	
32 ,, Reservoir	+	+	+·	+	−	−	10	10	+	+	+	+	+	−	5	12	
33 ,, Filtered	+	+	+·	−	−	−	10	10	+	+	+	+	+	−·	8	12	
34 ,, Tap	+	+	+	−·	−	−	10	10	+	+	+	+	+	−·	...	..	
35 ,, Tap	+	+	+·	+	−	−	10	10	+	+	+	+	+	+·	7	12	
36 Cocanada Canal	+	+	+	−	+	+	10	10	+	+	+	+	+	+·	11	12	
37 ,, Tank	+	+	+	+·	−	−	10	10	+	+	+	+	+·	−·	10	10	
38 ,, Filtered	+	+	+·	+	−	−	10	10	+	+	+	+	+	−	7	10	
39 ,, Tap	+	+	+	+	−	−	10	10	+	+	+	+	+	−·	0	12	
40 ,, Tap	+	+	+	+	−	−	10	10	+	+	+	+	+	+	2	12	
41 Conjeeveram	+	+	+	−·	−	−	10	10	+	+	+	+	+	−	0	12	
42 ,, Filtered	+	+	+	−	−	−	10	10	+	+	+	+	+	−·	0	12	
43 ,, Tap	+	+	+	−	−	−	10	10	+	+	+	+	+·	−·	0	12	
44 ,, Tap	+	+	+	−	−	−	10	10	+	+	+	+·	−	−	2	10	2 Lactose—Glucose—
45 Madura River	+	+	+	−	−	−	10	10	+	+	+	+	+	+	12	12	
46 ,, Filtered	+	+	+	−·	−	−	10	10	+	+	+	+	+	−	7	7	5 ,, ,,
47 ,, Tap	+	+	−	−	−	−	10	10	+	+	+	+	−·	−	7	8	4 ,, ,,
48 ,, Tap	+	+	+	−·	−	−	10	10	+	+	+	+	−·	−	6	6	6 ,, ,,
49 Nellore River	+	+	−	−	−	+	10	10	+	+	+	+	+	+	9	12	
50 ,, Filtered	+	−	−	−	−·	−	10	10	+	+	+	+·	+	+	5	12	
51 ,, Tap	+	−	−	+	+	−	10	10	+	+	+	+·	−	−	12	12	
52 ,, Tap	+	+	+	+	−	−	10	10	+	+	+	+	−	−	12	12	
53 ,, Tap	+	+	+	+	−·	−	10	10	+	+	+	+	+	−	0	12	
54 Tanjore	+	+	+	+	+	−	10	10	+	+	+	+·	−·	+	7	8	4 ,, ,,
55 ,, Filtered	+	+	+	−	−	−	10	10	+	+	+	−	−·	−	10	11	1 ,, ,,
56 ,, Tap	+	+	+	−	−	−	10	10	+	+	+	+	−·	−	12	12	
57 ,, Tap	+	+·	+	−	−	−	10	10	+	+	+	+	+	+	5	12	
58 Trichinopoly	+	+	+	−	+	+·	10	10	+	+	+	+	+	+	10	11	
59 ,, Filtered	+	+	+	+	−·	−	10	10	+	+	+	+	+·	−	3	5	7 ,, ,,
60 ,, Tap	+	+	+	+	−	−	10	10	+	+	+	+	+	−	4	11	1 ,, ,,
61 ,, Tap	+	+	+	+	−	−	10	10	+	+	+	+	+·	+·	9	11	1 ,, ,,
62 Tirupati	+	+	+	+	+	−	10	10	+	+	+	+	+	+	11	12	
63 ,, Well	+	+	+	+	−·	−	10	10	+	+	+	+	+	−	6	12	
64 ,, Filtered	+	+	+	−·	−	−	10	10	+	+	+	+	+	+	4	12	
65 ,, Tap	+	+	+	+	+	−	10	10	+	+	+	+	+	+	9	12	
66 ,, Tap	+	+	+	+	+·	−·	10	10	+	+	+	+	+	+	8	12	
67 Cuddapah	+	+	+	+	−·	−	10	10	+	+	+	+	+	+	4	12	
68 ,, Tap	+	+	+	+	−	−	10	10	+	+	+	+	−	−	4	12	
69 ,, Tap	+	+	+	+	−·	−	10	10	+	+	+	+	+	−	4	8	
70 Vizagapatam Lake	+	−	+	+	−·	−	10	10	+	+	+	+·	+	−	1	10	
71 ,, Filtered	+	+	+	·	−	−	10	10	+	+	+	+	−	−	2	10	
72 ,, Tap	+	+	+	−	−	−	10	10	+	+	+	+	−	−	0	10	
73 ,, Tap	+	+	+	−·	−	−	10	10	+	+	+	+	+	−	2	10	
74 ,, Tap	+	+	+	−·	−	−	10	10	+	+	+	+	+	−	0	10	
75 Dindigal Gallery	+	+	+	+	+	+	10	10	+	+	+	+	+	+	5	12	
76 ,, Well	+	+	+	+	+·	−	10	10	+	+	+	+	+	−	7	12	
77 ,, Tap	+	+	+	+	−·	−	10	10	+	+	+	+	+	+	7	11	1 ,, ,,
78 ,, Tap	+	+	+	−·	−	−	10	10	+	+	+	−	−	−	8	11	1 ,, ,,
Approximate Average	+	+	+	−·	−	−	770	770	+	+	+	+	+	−	447	842	35

Lactose +	Glucose +	770		100 %	(in lactose broth.)
Do. +	do.	+ 447		50·9%	(in glucose broth.)
Do. −	do.	+ 395		45·2%	do.
Do. +	do.	−	Nil.		
Do. −	do.	− 35		3·9%	do.

random (irrespective of their colour and appearance) and put through glucose and lactose broth. The results of these investigations are given in Table VI(*a*). Three tubes were inoculated for each step (3 1 c.c. ; 3 ·1 c.c., etc.) in the usual way. The + sign indicates that acid and gas were formed in all 3 tubes. If 2 out of the 3 gave acid and gas, asterisk is placed near the +; if 1 out of 3, a — sign with an asterisk, and if all 3 were negative a — sign is used to indicate this fact.

The following points should be carefully observed in the Table VI(*a*) :—

(1) That in practically every case a very much smaller quantity of the sample of water gives acid and gas in glucose than in lactose. If it were possible to make a sort of rough average out of these 77 samples, it would appear that in the lactose broth all dilutions up to 5 c.c. gave acid and gas, whereas in the glucose broth dilutions up to ·1 of a c.c. gave the reaction. If each sample is examined in turn, it will be found that out of these 77 only about 6 show that the number of organisms is approximately the same in both broths—(*vide* 15, 20, 36, 49, 58, 75). It may also be noted that all, except one, of these samples are taken from rivers, or irrigation canals.

It has been well known to water analysts for a very long time that what we may call " the acid and gas line " in lactose broth series is nearly always lower than in glucose broth.

(2) A careful scrutiny of the results obtained from the separate colonies is also necessary. In the first place, out of 770 colonies obtained from a lactose broth not a single one was glucose minus, that is to say, in this investigation all fæcal lactose fermenters ferment glucose. On the other hand, the organisms grown in a glucose broth and plated on a lactose medium show very different results. The figures are found at the bottom of the Table VI(*a*), *viz.*, about half of these organisms are glucose + lactose – . A very small percentage ferment neither glucose nor lactose ; no systematic study has been made of this small group up to the present. They are organisms which are obviously able to grow in a glucose broth without fermenting that sugar.

It should be observed that these samples were taken during July, August and September 1909. It is not maintained that the relative percentage of the three classes of organisms is constant at all times of the year. In fact, we shall show later on that this is not the case.

(3) Another point which should be noticed in connection with this table is that if the figures obtained from lake waters are compared with those of river waters, the relation between the two groups of bacilli is different in the two sources; thus glucose + lactose + form 42% of the organisms obtained from lakes, whereas in rivers and ground waters this class forms 76%. Therefore, it would appear that waters that have had considerable exposure to the sun contain more glucose + lactose – variety than do the others.

A second opportunity of making comparative investigation was afforded during a year's (1910) work that was carried out by myself on the Calcutta Water Supply. In this instance raw, settled and filtered waters were all put through glucose and lactose broth side by side. In this experiment, however, the 1 or ·1 c.c. dilution was generally used for obtaining the separate colonies. This fact must be carefully borne in mind, and the importance of it will be seen later on. Twenty colonies derived from a glucose broth, plated on a lactose solid medium, from each water sample, were put through glucose and lactose broth in order to ascertain the fermentative reactions of each. The results are given in Table VI (*b*), and are particularly instructive.

The same points that were mentioned in the former investigation are to be noted in this instance also, *viz.*, (1) the fact that much higher dilutions of the waters give acid and gas in glucose than in lactose; (2) the relation between the two groups of bacilli is shown in percentages for each month. It will be seen that they vary considerably month by month. Before going into this subject it should just be mentioned that after the end of October no rain fell during November, December, January and February, consequently we have a picture of results of self-purification of a river; except that in the case of the raw water samples the results are vitiated by the large amount of contamination which finds its way into

Table VI (*b*).

	RAW RIVER WATER.															
	HOWRAH BRIDGE.				KHURDAH RIVER.				BARRACKPORE.				PULTA INTAKE.			
	Lactose broth in c.c.	Glucose broth in c.c.	Out of 20 colonies.		Lactose broth in c.c.	Glucose broth in c.c.	Out of 20 colonies.		Lactose broth in c.c.	Glucose broth in c.c.	Out of 20 colonies.		Lactose broth in c.c.	Glucose broth in c.c.	Out of 20 colonies.	
			Glu+ Lac+	Glu+ Lac+			Glu+ Lac−	Glu+ Lac+			Glu+ Lac−	Glu+ Lac+			Glu+ Lac	Lac+ Glu+
OCTOBER.	...	...	...	...	..	...	...	...	·1	·01	Nil.	20	·1	·01	6	14
(Heavy rain.)	...	..	...	...	...	...	.	...	·1	·01	Nil.	20	·01	·01	10	10
	...	...		...		...	...	...	...	...	**0 p.c.**	**100 p.c.**	...	...	**40 p.c.**	**60 p.c.**
	·1	·01	Nil.	20	·01	·01	4	16	·1	·1	2	18	·01	·01	2	18
NOVEMBER.	·1	·01	3	17	·1	·1	3	17	·1	·01	Nil.	20	·1	·1	2	18
(River very muddy but no rain locally.)	·1	·01	4	16	·1	·01	7	13	·1	·01	6	14	1·0	·01	5	15
	·1	·01	9	11	1	·01	15	5	·1	·01	9	11	1·0	·01	16	4
	...		**20 p.c.**	**80 p.c**	...	...	**36·2 p.c.**	**63·8 p.c.**	...	..	**21·2 p.c**	**73·8 p.c**	...	...	**31·2 p.c.**	**68·8 p.c.**
	·1	·01	9	11	1·0	·1	8	12	·1	·1	16	4	·1	·1	10	10
DECEMBER.	·01	·1	Nil.	20	1	·1	17	3	1·0	·1	19	1	·1	·01	12	8
(River clearing.)	·01	·01	10	10	1·0	·01	11	9	·1	·01	13	7	·1	...	20	Nil.
	..		**63·1 p.c**	**68·3 p.c**	...	...	**60 p.c.**	**40 p.c.**	...	...	**80 p.c.**	**20 p.c.**	...	...	**70 p.c.**	**30 p.c.**
	·1	·01	11	9	1·0	·01	7	13	1	·01	16	4	·1	·01	15	5
	·01	·1	Nil.	20	1	·1	Nil.	20	1·0	·1	Nil.	20	·01	·01	Nil.	20
JANUARY.	·1	·1	11	9	·01	·01	20	Nil	·01	·01	13	7	·1	·01	7	13
(River clear.)	·01	·01	1	19	·01	·1	13	7	·1	·01	15	5	·01	·01	20	Nil.
	·1	·01	5	15	·1	·01	9	11	·01	·01	10	10	·01	·01	19	1
	.	.	**28 p.c.**	**72 p.c.**	...	..	**49 p.c**	**51 p.c.**	..	.	**54 p.c.**	**46 p.c.**	...	...	**61 p.c.**	**39 p.c.**
	·1	·01	2	18	·1	·01	16	4	·1	·01	14	6	1	·01	18	2
FEBRUARY.	·1	·01	18	2	1·0	·01	19	1	·1	·01	18	2	·1	·01	20	Nil.
(River clear.)	·1	·01	13	7	·1	·01	14	6	1	·01	11	9	1	·01	20	Nil.
	·01	·01	18	2	·01	·01	18	2	1	·01	18	2	·1	·01	17	3
	.	..	**63·7 p.c**	**36·3 p.c**	..	...	**83·7 p.c.**	**16·3 p.c.**	...	.	**76·2 p.c.**	**23·8 p.c**	.	...	**93·7 p.c**	**6·3 p.c.**

c.c.	Glucose.	Lactose.	c.c.	Glucose.	Lactose.	c.c.	Glucose.	Lactose.	c.c.	Glucose.	Lactose.
·01	87·5 p.c.	37·5 p.c.	·01	68·75 p.c.	25 p.c.	·01	77·7 p.c.	11·1 p.c.	·01	83·3 p.c.	27·7 p.c.
·1	12·5 p.c.	56·25 p.c.	·1	31·25 p.c	50 p.c.	·1	22·2 p.c.	77·7 p.c.	·1	16·6 p.c.	33·4 p.c.
1	...	6·25 p.c.	1	...	25 p.c.	1	...	11·1 p.c.	1	...	38·8 p.c.
5	...	..	5	...	...	5	...	...	5	...	...
10	...	...	10	...	...	10	...	—	10	...	...
20	...	—	20	...	...	20	...	...	20	...	...
60	...	...	60	...	...	60	...	..	60	...	...

	SETTLED WATER.				FILTERED WATER.											
	PULTA.				PULTA.				TALLAH.				WELLINGTON SQUARE.			
	Lactose broth in c.c.	Glucose broth in c.c.	Out of 20 colonies.		Lactose broth in c.c.	Glucose broth in c.c.	Out of 20 colonies.		Lactose broth in c.c.	Glucose broth in c.c.	Out of 20 colonies.		Lactose broth in c.c.	Glucose broth in c.c.	Out of 20 colonies.	
			Glu+ Lac−	Glu+ Lac+			Glu+ Lac−	Glu+ Lac+			Glu+ Lac−	Glu+ Lac+			Glu+ Lac−	Glu + Lac +
OCTOBER.	·01	·01	10	10	5	1	20	Nil	5	1	Nil	20	5	1	7	13
(Heavy rain.)	·01	·01	10	10	10	5	...	20	5	1	3	17	1	·1	5	15
	...	...	**50 p.c.**	**50 p.c.**	...	...	**50 p.c.**	**50 p.c.**	...	...	**7·5 p.c.**	**92·5 p.c.**	..	...	**30 p.c.**	**70 p.c.**
	1·0	·01	Nil	20	Nil	·01	11	9	1	1	1	19	1	1	Nil	20
NOVEMBER.	·1	·01	1	19	0	5	3	17	5	1	Nil	20	1	1	Nil	20
(River very muddy but no rain locally.)	·5	·01	10	10	10	10	19	1	1	·01	11	9	5	1	2	18
	·1	·01	2	18	Nil	1	6	14	Nil	5	16	4	1	·1	9	11
	...	...	**16·2 p.c**	**83·8 p.c.**	...	...	**48·7 p.c.**	**51·3 p.c**	...	...	**35 p.c.**	**65 p.c.**	...	...	**13·7 p.c.**	**86·3 p.c.**
	1	·01	18	2	Nil	5	20	Nil	5	1	17	3	1	·1	4	16
DECEMBER.	5	·01	14	6	Nil	5	17	3	10	1	16	4	5	1	Nil	20
(River clearing.)	5	·1	19	1	Nil	5	18	2	5	·1	18	2	·1	·01	2	Nil
	...	...	**85 p.c.**	**15 p.c.**	—	...	**91·6 p.c.**	**8·4 p.c**	..	...	**85 p.c.**	**15 p.c.**			**40 p.c.**	**60 p.c.**
	5	·01	13	7	5	5	20	Nil.	5	1	19	1	5	1	18	2
	1	1	Nil.	20	Nil.	10		...	Nil.	10	20	Nil.	5	1	20	Nil.
JANUARY.	·1	·01	20	Nil.	Nil	10	20	Nil.	10	1	19	1	5	1	20	Nil.
(River clear.)	1	·01	18	2	10	1	20	Nil.	Nil.	1	20	Nil.	10	1	20	Nil.
	1	·1	20	Nil.	...	...	...	...	...	...	..	...	...	...	...	..
	.	.	**71 p.c.**	**29 p.c.**	...		**100 p.c.**	**0 p.c.**	...	...	**97·5 p.c.**	**2·5 p.c.**	..	...	**97·5 p.c**	**2·5 p.c.**
	5	·1	20	Nil.	Nil.	5	19	1	10	1	20	Nil.	5	1	20	Nil.
FEBRUARY.	10	·1	20	Nil.	Nil.	5	20	Nil.	Nil.	1	16	4	10	1	16	4
(River clear.)	5	·1	19	1	Nil.	Nil.	...	...	60	5	...	...	20	5	19	1
	5	·1	20	Nil.	Nil.	10	20	Nil.	Nil.	1	20	Nil.	10	1	20	Nil.
	...	...	**98·7 p.c.**	**1·3 p.c.**	...	...	**98·3 p.c.**	**1·7 p.c.**	...	.	**93·3 p.c.**	**6·7 p.c.**	.	..	**93·7 p.c**	**6·3 p.c.**

c.c.	Glucose.	Lactose.	c.c.	Glucose.	Lactose.	c.c.	Glucose.	Lactose.	c.c.	Glucose.	Lactose.
·01	61·1 p.c.	11·1 p.c.	·01	6 p.c.	0 p.c.	·01	6 p.c.	0 p.c.	·01	6 p.c.	0 p.c.
·1	33·3 p.c.	22·2 p.c.	·1	0 p.c.	0 p.c.	·1	6 p.c.	0 p.c.	1	18 p.c.	6 p.c.
1	5·5 p.c.	22·2 p.c.	1	18 p.c.	0 p.c.	1	72 p.c.	12 p.c.	1	78 p.c.	30 p.c.
5	...	38·8 p.c.	5	48 p.c.	12 p.c.	5	12 p.c.	36 p.c.	5	0 p.c	42 p.c.
10	...	5·5 p.c.	10	32 p.c.	24 p.c.	10	6 p.c.	18 p.c.	10	...	18 p.c.
20	...	...	20	0 p.c.	0 p.c.	20	0 p.c.	0 p.c.	20	...	6 p.c.
60	...	...	60	6 p.c.	66 p.c.	60	0 p.c.	36 p.c.	60	...	0 p.c.

the river along the banks. The main point which is demonstrated by practically all these samples is that in rainy weather, when the river is muddy and contamination gross, the class glucose + lactose + is extremely common, and 4 months later, in February, when the river is clear, the reverse is the case. Particular attention is drawn to the comparison between the 3 filtered waters of Pulta, Tallah and Wellington Square in the months of November and December. Note the great prevalence of the class glucose + lactose + in Wellington Square, as compared with the Pulta and Tallah samples. The explanation of this fact is that highly polluted subsoil water was making its way into the Wellington Square reservoir during this period. This was proved by other bacteriological evidence, and by inspection of the reservoir. As the level of the subsoil water sank, the pollution ceased, so the relation between these two classes of organisms (glucose + lactose +, and glucose + lactose –) assumes what may be called the normal during the months of January and February.

TABLE VI (*c*).

After Houston.

Number of typical B. coli per 100 *coli-like microbes of all sorts.*

1908-9. Month.	THAMES.		LEE.		NEW RIVER.	
	Raw. %	Filtered. %	Raw. %	Filtered. %	Raw. %	Filtered. %
Averages, 1907-8	80·5	54·9	84·2	45·7	79·4	54·6
April, 1908 ..	81·8	45·3	87·2	25·0 (1 out of 4)	72·9	26·3
May ..	71·1	30·6	79·7	20·0	80·3	23·7
June ..	68·7	30·7	73·6	51·6	66·1	23·4
July ..	69·8	37·9	88·5	29·0	76·0	38·6
August ..	80·0	34·1	79·5	14·3	78·5	39·3
September ..	71·4	26·2	83·1	30·0	74·6	60·0

TABLE VI(*c*)—(*contd.*)

1908-9. Month.	THAMES.		LEE.		NEW RIVER.	
	Raw. %	Filtered. %	Raw. %	Filtered. %	Raw %	Filtered. %
October ..	78·2	43·8	85·7	47·0	81·9	61·9
November ..	76·1	28·5	83·7	16·6	81·4	60·8
December ..	83·7	50·7	80·3	66·6	73·2	52·8
January, 1909 ..	86·6	68·6	85·7	50·0	75·6	60·0
February ..	83·9	50·8	86·6	20·0 (1 out of 5)	63·9	18·7
March ..	83·7	60·1	92·6	64·7	79·6	29·4
1909-10.						
Averages, 1908-9	**77·3**	**42·8**	**83·9**	**39·2**	**75·8**	**43·0**
April, 1909 ..	85·4	28·3	92·3	*Nil*	82.3	*Nil*
May ..	72·3	14·0	82·3	28·5 (2 out of 7)	81·6	37·5
June ..	85·1	22·0	82·5	*Nil.*	61·3	40·0
July ..	82·5	20·7	82·1	22·7	81·5	27·6
August ..	68·0	25·6	67·0	14·7	71·9	8·6
September ..	74·0	17·8	74·6	*Nil.*	64·5	17·2
October ..	80·3	23·1	79·3	44·4	74·1	33·3
November ..	88·0	46·8	85·3	33·3	69·8	44·9
December ..	87·6	57·7	82·6	37·5	77·0	65·2
January, 1910 ..	87·6	54·2	90·6	81·8	82·5	35·1
February ..	85·7	59·4	94·0	60·0	84·5	45·1
March ..	86·2	42·8	76·2	*Nil.*	62·0	20·8
Averages, 1909-10	**82·3**	**32·6**	**82·2**	**28·2**	**73·9**	**37·8**

It seems hardly necessary to cite further evidence, but one other instance may be mentioned. The work of Houston shows that exactly the same state of affairs exists, to a less extent, in England. The tables taken from his reports, 1909, 1910 and

1911, demonstrate these points to a remarkable degree. Note that in Table No. VI(*c*) the mid-summer figure in raw and filtered water always shows a less percentage of "true coli" (lactose + glucose + indol +) than the figures of the winter months. Further, Table VI(*d*) for the 3 years taken from Houston's report also demonstrates the fact that water *after storage* contains a very much higher percentage of "non-typical coli," that is (lactose − glucose +) than do unstored or raw waters.

TABLE VI(*d*).

After Houston.

TYPE OF B. COLI.

1909.		*Raw* Waters.		*Filtered* Waters.*	
Type of B. coli.		Out of 2,595 specimens of B. coli isolated from 696 samples of *raw* water, the proportion between the typical and non-typical races of B. coli, expressed as percentages, was as follows :—		Out of 3,164 specimens of B. coli isolated from 7,081 samples of *filtered* water (including Kent and Lee Valley unfiltered well water), the proportion between the typical and non-typical races of B. coli, expressed as percentages, was as follows :—	
Typical B. coli (+lactose ; +indol) ...		79·4		42·8	
Non-typical B. coli	(+lactose ;—indol)	11·9	20·5	25·2	57·1
	(—lactose ; + glucose)	8 6		31·9	

1910.		*Raw* Waters.		*Filtered* Waters.*	
Type of B. coli.		Out of 2.427 specimens of B. coli isolated from 2612 samples of *raw* water, the proportion between the typical and non-typical races of B. coli, expressed as percentages, was as follows :—		Out of 3.934 specimens of B. coli isolated from 6,219 samples of *filtered* water (including Kent and Lee Valley unfiltered well water), the proportion between the typical and non-typical races of B. coli, expressed as percentages, was as follows :—	
Typical B. coli (+lactose ; +indol) .		80·0		35·0	
Non-typical B. coli	(+lactose ; +indol) ...	8·7	19·9	14·2	64·9
	(—lactose ; + glucose)	11·2		50·7	

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* Filtered waters all have more or less storage and exposure to sun.

1911.		*Raw* Waters.		*Filtered* Waters.*	
Type of B. coli.		Out of 2,635 specimens of B. coli isolated from 741 samples of *raw* water, the proportion between the typical and non-typical races of B. coli, expressed as percentages, was as follows :—		Out of 3,522 specimens of B. coli isolated from 7,146 samples of *filtered* water (including Kent and Lee Valley unfiltered well water), the proportion between the typical and non-typical races of B. coli, expressed as percentages, was as follows :—	
Typical B. coli (+ lactose ; — indol) ...		—	... 81·8	—	... 49·8
Non-typical B. coli	(+ lactose ; + indol)...	9·3	... 18·1	19·8	... 50·2
	(—lactose ; + glucose)	8·8		30·4	

* Filtered waters all have more or less storage and exposure to sun.

Considering this mass of evidence we may consider that the following points are fairly certain :—

(*i*) that the acid and gas line in glucose medium is higher than in lactose in ordinary natural water ;

(*ii*) that the relation between the two classes of organisms varies at different times of the year ;

(*iii*) that the relation between the two classes of organisms seems to be in some way connected with the amount of storage, or exposure to the sun.

Further evidence is necessary before this last point can be accepted as true, and we must enquire into the explanation of these facts.

We may commence the enquiry by the question—How do these facts tally with the work on fresh fæces given previously? In Chapter III we have shown that the particular class of organism, *viz.*, glucose + lactose –, is, as far as one can tell from a very extended investigation on fresh fæces, extremely rare ; they represent only 5% or less of the total coliform organism. Yet within a few weeks of the date of addition of fæces to water the picture is entirely reversed ; the lactose + glucose + represent 5% of all fæcal organisms (as shown by Table VI(*b*)) and the glucose + lactose – now account for the remaining 95%. How has this change come about, and what is its significance?

TABLE VI (*e*).

COMPARATIVE RESULTS IN GLUCOSE AND LACTOSE BROTH IN AN ARTIFICIALLY POLLUTED POND.

Date.	EXPERIMENT I.				Date.	EXPERIMENT II.				Date.	EXPERIMENT III.				Date.	EXPERIMENT IV.			
	Surface.		Bottom.			Surface.		Bottom.			Surface.		Bottom.			Surface.		Bottom.	
	Glucose.	Lactose.	Glucose.	Lactose.		Glucose.	Lactose.	Glucose.	Lactose.		Glucose.	Lactose.	Glucose.	Lactose.		Glucose.	Lactose.	Glucose.	Lactose.
16-12-10	·01	·01	·01	·01	..	..	..	..	..	4-4-11	·01	·01	·01	·01	2-5-11	·01	·01	·01	·01
16-12-10	·01	·01	·01	·1	20-2-11	·01	·01	·01	·01	4-4-11	·1	1	·01	1	2-5-11	·1	1	·01	·1
17-12-10	·1	1	·1	1	21-2-11	·1	·1	·01	·01	5-4-11	1	5	·1	1	3-5-11	·1	1	·01	1
18-12-10	·1	1	·1	1	22-2-11	·1	·1	·1	·1	7-4-11	1	5	·1	1	4-5-11	·1	1	·01	1
19-12-10	·01	·1	·1	·1	23-2-11	·1	5	·1	1	10-4-11	·1	5	·01	1	5-5-11	1	5	·01	1
20-12-10	·01	1	·01	·1	27-2-11	1	5	1	5	21-4-11	·1	1	·01	·1	7-5-11	1	5	·1	1
21-12-10	·1	1	·01	·01	1-3-11	1	1	1	5						8-5-11	·1	1	·01	·1
22-12-10	·1	1	..	..	7-3-11	·5	1	..	..	Experiment stopped on account of heavy downpour of rain 1·5 m.					10-5-11	·1	5	·01	·1
26-12-10	·01	1	·01	·01	27-3-11	·1	5	·01	1						11-5-11	·1	5	·01	1
28-12-10	·1	5	·01	·1	..	..	..	..	..						12-5-11	·1	5	..	..
30-12-10	·01	1	·01	·1	..	..	..	..	..						13-5-11	1	5	..	..
31-12-10	·1	1	·1	1	..	..	..	..	..						24-5-11	1	10	·01	1
2-1-11	·1	1	·1	1	..	..	..	..	..						26-5-11	1	5	·01	1
3-1-11	·1	1	·01	1	..	..	..	..	..										
6-1-11	·01	1	·1	1	..	..	..	..	..										
18-1-11	1	5	·1	1	..	..	..	..	..										
28-1-11	·1	5	·01	1	..	..	..	..	..										

The change between the two extremes is of course a very violent one, but it is easily demonstrated that in nature the process is a gradual one. In order further to investigate the change described above, three separate investigations were undertaken :—

1. Situated in the compound of the laboratory is a large tank about an acre and a half in extent. This, during the hot weather months, usually contains quite a good water, that is to say, a surface sample contains no lactose fermenters in 5 c.c. Generally, however, the glucose + lactose – are rather numerous. Into this large pond was thrown two or three tubs full of nightsoil, cowdung, effluent, etc., care being taken to distribute the material over the water and to make a mixture. Analyses were made on succeeding days after it had been polluted in this way. Four separate investigations of this nature were carried out and the results are almost identical in every case. Several other points were also investigated during these experiments. Table No. VI(*e*) gives the details of experiment, samples being taken both from the surface and from the bottom of the tank.

Before discussing the results it is necessary to point out certain obvious objections to these experiments. First, we did not begin with sterile water, and therefore it is impossible really to be quite sure that the organisms which remain to the end of the experiment were not those originally present in the tank. Secondly, the gap between ·1 and ·01 in our estimation is too wide to show delicate shades of difference between the acid and gas line in both media. Thirdly, the fæces used were obtained in the hot weather when very few varieties of organisms are normally present. The bacilli isolated at this time have a tendency to belong to the resistant varieties.

Making due allowance for these objections the following points are demonstrated by the table :—

(1) That on all occasions when the water has been recently polluted, acid and gas line in the glucose and lactose medium is the same in both cases.

(2) Within a very few days there is a very rapid decrease in all the fæcal organisms irrespective of class.

(3) While the lactose fermenting organisms go on decreasing in number, the results show that the class glucose + lactose – remain stationary, or increase.

(4) That on the whole fæcal organisms of all kinds are more plentiful at the bottom than on the surface.

II. On account of the objections that could be raised to the above series of experiments, we attempted to prove the same point on a large natural sheet of water which only required a heavy downpour of rain in order to pollute it. The place chosen was the Red Hills Lake in Madras which is about 14 miles in circumference. For reasons which need not be given in detail here, a holiday of a few weeks' duration was all the time that could be given to this work, and during the period only one shower fell. But the results are fairly satisfactory. This experiment will be referred to later on, as other points of very great interest are also demonstrated. The following points should be observed in Table VI(*f*).

(*i*) Note the analysis before the fall of rain ; the great increase in the number of organisms of all kinds due to the rain ; the coincidence of the acid and gas line after pollution.

(*ii*) Observe the very rapid fall in number of bacilli in all samples for about 4 or 5 days and the purity of the surface sample in No. 7.

(*iii*) From about this point the glucose + lactose – start to increase, and do so fairly steadily throughout the experiment.

(*iv*) Note also a short similar increase in the glucose + lactose +, this will be discussed fully later on ; it is due to one variety of the class growing in the water at certain times.

Besides these points it must also be stated that an analysis on the 20th showed nothing but bacillus *P* ; on the 21st and 22nd other non-lactose fermenters were mixed with these ; but from 23rd onwards only bacillus *P* could be isolated.

III. A third series of investigations have been carried out on great rivers in Bengal during the rains and dry weather, in order to observe what takes place during the process of clearing when the rains are over. A series of very careful weekly, or fortnightly, analyses have been taken of the river Ganges at various points. It is not an easy matter to demonstrate by

Madras Red Hills Lake Waters. September 1911. TABLE VI (*f*).

1

Surface .. 20-9-11.

	Glucose.		Lactose.	
	+	—	+	—
1 .. 20	1	*Nil*	1	*Nil*
2 .. 10	2	*Nil*	2	*Nil*
3 .. 5	3	*Nil*	3	*Nil*
3 .. 1	3	*Nil*	1	2
3 .. ·1	3	*Nil*	*Nil*	3
3 .. ·05	3	*Nil*	1	2
3 .. ·03	*Nil*	3	*Nil*	3
3 .. ·01	*Nil*	3	*Nil*	3
3 .. ·005	*Nil*	3	*Nil*	3
3 .. ·003	*Nil*	3	*Nil*	3
3 .. ·001	*Nil*	3	*Nil*	3
note.	·05		5	

Typical dry weather results

Bottom .. 20-9-11.

	Glucose.		Lactose.	
	+	—	+	—
1 .. 20	1	*Nil*	1	*Nil*
2 .. 10	2	*Nil*	2	*Nil*
3 .. 5	3	*Nil*	3	*Nil*
3 .. 1	3	*Nil*	2	1
3 .. ·1	3	*Nil*	*Nil*	3
3 .. ·05	3	*Nil*	1	2
3 .. ·03	1	2	*Nil*	3
3 .. ·01	*Nil*	3	*Nil*	3
3 .. ·005	*Nil*	3	*Nil*	3
3 .. ·003	*Nil*	3	*Nil*	3
3 .. ·001	*Nil*	3	*Nil*	3
	·05		1	

2

Surface .. 21-9-11.

Rain fell here.

	Glucose.		Lactose.	
	+	—	+	—
1 .. 20	1	*Nil*	1	*Nil*
2 .. 10	2	*Nil*	2	*Nil*
3 .. 5	3	*Nil*	3	*Nil*
3 .. 1	3	*Nil*	3	*Nil*
3 .. ·1	3	*Nil*	3	*Nil*
3 .. ·05	3	*Nil*	3	*Nil*
3 .. ·03	3	*Nil*	2	1
3 .. ·01	3	*Nil*	1	2
3 .. ·005	*Nil*	3	*Nil*	3
3 .. ·003	*Nil*	3	*Nil*	3
3 .. ·001	*Nil*	3	*Nil*	3
	·01		·03	

Bottom .. 21-9-11.

	Glucose.		Lactose.	
	+	—	+	—
1 .. 20	1	*Nil*	1	*Nil*
2 .. 10	2	*Nil*	2	*Nil*
3 .. 5	3	*Nil*	3	*Nil*
3 .. 1	3	*Nil*	3	*Nil*
3 .. ·1	3	*Nil*	3	*Nil*
3 .. ·05	3	*Nil*	3	*Nil*
3 .. ·03	3	*Nil*	3	*Nil*
3 .. ·01	3	*Nil*	3	*Nil*
3 .. ·005	*Nil*	3	*Nil*	3
3 .. ·003	*Nil*	3	*Nil*	3
3 .. ·001	*Nil*	3	*Nil*	3
	·01		·01	

3

Surface .. 22-9-11.

	Glucose.		Lactose.	
	+	—	+	—
1 .. 20	1	*Nil*	1	*Nil*
2 .. 10	2	*Nil*	2	*Nil*
3 .. 5	3	*Nil*	3	*Nil*
3 .. 1	3	*Nil*	3	*Nil*
3 .. ·1	3	*Nil*	3	*Nil*
3 .. ·05	3	*Nil*	3	*Nil*
3 .. ·03	3	*Nil*	3	*Nil*
3 .. ·01	3	*Nil*	3	*Nil*
3 .. ·005	3	*Nil*	3	*Nil*
3 .. ·003	3	*Nil*	3	*Nil*
3 .. ·001	2	1	1	2
	·001		·003	

Bottom .. 22-9-11.

	Glucose.		Lactose.	
	+	—	+	—
1 .. 20	1	*Nil*	1	*Nil*
2 .. 10	2	*Nil*	2	*Nil*
3 .. 5	3	*Nil*	3	*Nil*
3 .. 1	3	*Nil*	3	*Nil*
3 .. ·1	3	*Nil*	3	*Nil*
3 .. ·05	3	*Nil*	3	*Nil*
3 .. ·03	3	*Nil*	3	*Nil*
3 .. ·01	3	*Nil*	3	*Nil*
3 .. ·005	3	*Nil*	3	*Nil*
3 .. ·003	3	*Nil*	2	1
3 .. ·001	*Nil*	3	*Nil*	3
	.003		·003	

4

Surface .. 23-9-11.

	Glucose.		Lactose.	
	+	—	+	—
1 .. 20	1	*Nil*	1	*Nil*
2 .. 10	2	*Nil*	2	*Nil*
3 .. 5	3	*Nil*	3	*Nil*
3 .. 1	3	*Nil*	3	*Nil*
3 .. ·1	3	*Nil*	3	*Nil*
3 .. ·05	3	*Nil*	2	1
3 .. ·03	3	*Nil*	2	1
3 .. ·01	3	*Nil*	2	1
3 .. ·005	2	1	*Nil*	3
3 .. ·003	1	2	*Nil*	3
3 .. ·001	*Nil*	3	*Nil*	3
	·005		·01	

Bottom .. 23-9-11(?).

	Glucose.		Lactose.	
	+	—	+	—
1 .. 20	1	*Nil*	1	*Nil*
2 .. 10	2	*Nil*	2	*Nil*
3 .. 5	3	*Nil*	3	*Nil*
3 .. 1	3	*Nil*	2	1
3 .. ·1	3	*Nil*	*Nil*	3
3 .. ·05	2	1	*Nil*	3
3 .. ·03	*Nil*	3	*Nil*	3
3 .. ·01	*Nil*	3	*Nil*	3
3 .. ·005	*Nil*	3	*Nil*	3
3 .. ·003	*Nil*	3	*Nil*	3
3 .. ·001	*Nil*	3	*Nil*	3
	·05		1	

Madras Red Hills Lake Waters. September 1911. TABLE VI (*f*). (*contd.*)

5

Surface .. 24-9-11.

		Glucose.		Lactose.	
		+	−	+	−
1 ..	20	1	*Nil*	1	*Nil*
2 ..	10	2	*Nil*	2	*Nil*
3 ..	5	3	*Nil*	3	*Nil*
3 ..	1	3	*Nil*	3	*Nil*
3 ..	·1	3	*Nil*	*Nil*	3
3 ..	·05	*Nil*	3	*Nil*	3
3 ..	·03	*Nil*	3	*Nil*	3
3 ..	·01	*Nil*	3	*Nil*	3
3 ..	·005	*Nil*	3	*Nil*	3
3 ..	·003	*Nil*	3	*Nil*	3
3 ..	·001	*Nil*	3	*Nil*	3
		·1		1	

6

Surface .. 25-9-11.

		Glucose.		Lactose.	
		+	−	+	−
1 ..	20	1	*Nil*	1	*Nil*
2 ..	10	2	*Nil*	2	*Nil*
3 ..	5	3	*Nil*	2	1
3 ..	1	3	*Nil*	*Nil*	3
3 ..	·1	*Nil*	3	*Nil*	3
3 ..	·05	*Nil*	3	*Nil*	3
3 ..	·03	*Nil*	3	*Nil*	3
3 ..	·01	*Nil*	3	*Nil*	3
3 ..	·005	*Nil*	3	*Nil*	3
3 ..	·003	*Nil*	3	*Nil*	3
3 ..	·001	*Nil*	3	*Nil*	3
		1		5	

7

Surface .. 26-9-11.

		Glucose.		Lactose.	
		+	−	+	−
1 ..	20	1	*Nil*	*Nil*	1
2 ..	10	2	*Nil*	1	1
3 ..	5	3	*Nil*	*Nil*	3
3 ..	1	*Nil*	3	*Nil*	3
3 ..	·1	*Nil*	3	*Nil*	3
3 ..	·05	*Nil*	3	*Nil*	3
3 ..	·03	*Nil*	3	*Nil*	3
3 ..	·01	*Nil*	3	*Nil*	3
3 ..	·005	*Nil*	3	*Nil*	3
3 ..	·003	*Nil*	3	*Nil*	3
3 ..	·001	*Nil*	3	*Nil*	3
		5		60	

8

Surface .. 27-9-11.

		Glucose.		Lactose.	
		+	−	+	−
1 ..	20	1	*Nil*	1	*Nil*
2 ..	10	2	*Nil*	2	*Nil*
3 ..	5	3	*Nil*	3	*Nil*
3 ..	1	3	*Nil*	*Nil*	3
3 ..	·1	1	2	*Nil*	3
3 ..	·05	1	2	*Nil*	3
3 ..	·03	*Nil*	3	*Nil*	3
3 ..	·01	*Nil*	3	*Nil*	3
3 ..	·005	*Nil*	3	*Nil*	3
3 ..	·003	*Nil*	3	*Nil*	3
3 ..	·001	*Nil*	3	*Nil*	3
		1		5	

Bottom .. 24-9-11.

		Glucose.		Lactose.	
		+	−	+	−
1 ..	20	1	*Nil*	1	*Nil*
2 ..	10	2	*Nil*	2	*Nil*
3 ..	5	3	*Nil*	3	*Nil*
3 ..	1	3	*Nil*	3	*Nil*
3 ..	·1	3	*Nil*	1	2
3 ..	·05	*Nil*	3	1	2
3 ..	·03	1	2	*Nil*	3
3 ..	·01	*Nil*	3	*Nil*	3
3 ..	·005	*Nil*	3	*Nil*	3
3 ..	·003	*Nil*	3	*Nil*	3
3 ..	·001	*Nil*	3	*Nil*	3
		·1		1	

Bottom .. 25-9-11.

		Glucose.		Lactose.	
		+	−	+	−
1 ..	20	1	*Nil*	1	*Nil*
2 ..	10	2	*Nil*	2	*Nil*
3 ..	5	3	*Nil*	3	*Nil*
3 ..	1	3	*Nil*	1	2
3 ..	·1	2	1	*Nil*	3
3 ..	·05	1	2	*Nil*	3
3 ..	·03	*Nil*	3	*Nil*	3
3 ..	·01	*Nil*	3	*Nil*	3
3 ..	·005	*Nil*	3	*Nil*	3
3 ..	·003	*Nil*	3	*Nil*	3
3 ..	·001	*Nil*	3	*Nil*	3
		·1		5	

Bottom .. 26-9-11.

		Glucose.		Lactose.	
		+	−	+	−
1 ..	20	1	*Nil*	1	*Nil*
2 ..	10	2	*Nil*	2	*Nil*
3 ..	5	3	*Nil*	*Nil*	3
3 ..	1	3	*Nil*	*Nil*	3
3 ..	·1	3	*Nil*	*Nil*	3
3 ..	·05	1	2	*Nil*	3
3 ..	·03	*Nil*	3	*Nil*	3
3 ..	·01	*Nil*	3	*Nil*	3
3 ..	·005	*Nil*	3	*Nil*	3
3 ..	·003	*Nil*	3	*Nil*	3
3 ..	·001	*Nil*	3	*Nil*	3
		·1		10	

Bottom .. 27-9-11.

		Glucose.		Lactose.	
		+	−	+	−
1 ..	20	1	*Nil*	1	*Nil*
2 ..	10	2	*Nil*	2	*Nil*
3 ..	5	3	*Nil*	3	*Nil*
3 ..	1	3	*Nil*	3	*Nil*
3 ..	·1	3	*Nil*	*Nil*	3
3 ..	·05	3	*Nil*	*Nil*	3
3 ..	·03	2	1	*Nil*	3
3 ..	·01	*Nil*	3	*Nil*	3
3 ..	·005	*Nil*	3	*Nil*	3
3 ..	·003	*Nil*	3	*Nil*	3
3 ..	·001	*Nil*	3	*Nil*	3
		·03		1	

Madras Red Hills' Lake Waters. September and October 1911. TABLE VI(*f*)—(contd.)

9

Surface .. 28-9-11.

		Glucose.		Lactose.	
		+	−	+	−
1 ..	20	1	*Nil*	1	*Nil*
2 ..	10	2	*Nil*	2	*Nil*
3 ..	5	3	*Nil*	3	*Nil*
3 ..	1	3	*Nil*	3	*Nil*
3 ..	·1	3	*Nil*	2	1
3 ..	·05	1	2	*Nil*	3
3 ..	·03	*Nil*	3	*Nil*	3
3 ..	·01	*Nil*	3	*Nil*	3
3 ..	·005	*Nil*	3	*Nil*	3
3 ..	·003	*Nil*	3	*Nil*	3
3 ..	·001	*Nil*	3	*Nil*	3
		·1		·1	

10

Surface .. 29-9-11.

		Glucose.		Lactose.	
		+	−	+	−
1 ..	20	1	*Nil*	1	*Nil*
2 ..	10	2	*Nil*	2	*Nil*
3 ..	5	3	*Nil*	2	1
3 ..	1	3	*Nil*	*Nil*	3
3 ..	·1	3	*Nil*	*Nil*	3
3 ..	·05	*Nil*	3	*Nil*	3
3 ..	·03	*Nil*	3	*Nil*	3
3 ..	·01	*Nil*	3	*Nil*	3
3 ..	·005	*Nil*	3	*Nil*	3
3 ..	·003	*Nil*	3	*Nil*	3
3 ..	·001	*Nil*	3	*Nil*	3
		·1		5	

11

Surface .. 30-9-11.

		Glucose.		Lactose.	
		+	−	+	−
1 ..	20	1	*Nil*	1	*Nil*
2 ..	10	2	*Nil*	2	*Nil*
3 ..	5	3	*Nil*	3	*Nil*
3 ..	1	3	*Nil*	3	*Nil*
3 ..	·1	3	*Nil*	*Nil*	3
3 ..	·05	2	1	*Nil*	3
3 ..	·03	1	2	*Nil*	3
3 ..	·01	*Nil*	3	*Nil*	3
3 ..	·005	*Nil*	3	*Nil*	3
3 ..	·003	*Nil*	3	*Nil*	3
3 ..	·001	*Nil*	3	*Nil*	3
		·05		·1	

12

Surface .. 5-10-11.

		Glucose.		Lactose.	
		+	−	+	−
1 ..	20	1	*Nil*	*Nil*	1
2 ..	10	2	*Nil*	*Nil*	2
3 ..	5	3	*Nil*	*Nil*	3
3 ..	1	3	*Nil*	*Nil*	3
3 ..	·1	3	*Nil*	*Nil*	3
3 ..	·05	*Nil*	3	*Nil*	3
3 ..	·03	*Nil*	3	*Nil*	3
3 ..	·01	1	2	*Nil*	3
3 ..	·005	*Nil*	3	*Nil*	3
3 ..	·003	*Nil*	3	*Nil*	3
3 ..	·001	*Nil*	3	*Nil*	3
		·1		60	

Bottom .. 28-9-11.

		Glucose.		Lactose.	
		+	−	+	−
1 ..	20	1	*Nil*	1	*Nil*
2 ..	10	2	*Nil*	2	*Nil*
3 ..	5	3	*Nil*	3	*Nil*
3 ..	1	3	*Nil*	3	*Nil*
3 ..	·1	3	*Nil*	3	*Nil*
3 ..	·05	2	1	*Nil*	3
3 ..	·03	2	1	*Nil*	3
3 ..	·01	2	1	*Nil*	3
3 ..	·005	*Nil*	3	*Nil*	3
3 ..	·003	*Nil*	3	*Nil*	3
3 ..	·001	*Nil*	3	*Nil*	3
		·01		·1	

Bottom .. 29-9-11.

		Glucose.		Lactose.	
		+	−	+	−
1 ..	20	1	*Nil*	1	*Nil*
2 ..	10	2	*Nil*	2	*Nil*
3 ..	5	3	*Nil*	3	*Nil*
3 ..	1	3	*Nil*	3	*Nil*
3 ..	·1	3	*Nil*	3	*Nil*
3 ..	·05	3	*Nil*	3	*Nil*
3 ..	·03	3	*Nil*	1	2
3 ..	·01	1	2	1	2
3 ..	·005	1	2	*Nil*	3
3 ..	·003	1	2	*Nil*	3
3 ..	·001	*Nil*	3	*Nil*	3
		·03		·05	

Bottom .. 30-9-11.

		Glucose.		Lactose.	
		+	−	+	−
1 ..	20	1	*Nil*	1	*Nil*
2 ..	10	2	*Nil*	2	*Nil*
3 ..	5	3	*Nil*	3	*Nil*
3 ..	1	3	*Nil*	3	*Nil*
3 ..	·1	3	*Nil*	3	*Nil*
3 ..	·05	3	*Nil*	1	2
3 ..	·03	3	*Nil*	1	2
3 ..	·01	2	1	*Nil*	3
3 ..	·005	2	1	*Nil*	3
3 ..	·003	1	2	*Nil*	3
3 ..	·001	*Nil*	3	*Nil*	3
		·005		·1	

Bottom .. 5-10-11.

		Glucose.		Lactose.	
		+	−	+	−
1 ..	20	1	*Nil*	1	*Nil*
2 ..	10	2	*Nil*	2	*Nil*
3 ..	5	3	*Nil*	1	2
3 ..	1	3	*Nil*	*Nil*	3
3 ..	·1	1	2	*Nil*	3
3 ..	·05	*Nil*	3	*Nil*	3
3 ..	·03	*Nil*	3	*Nil*	3
3 ..	·01	*Nil*	3	*Nil*	3
3 ..	·005	*Nil*	3	*Nil*	3
3 ..	·003	*Nil*	3	*Nil*	3
3 ..	·001	*Nil*	3	*Nil*	3
		1		10	

figures the results of the analyses, but some selected samples are given. In every case when the river is in high flood, or rain is actually falling or has fallen recently, the acid and gas line in the two media is very close together, *vide* samples 1-12 Table VI (*g*). Conversely, in the dry weather the line separates considerably, and bacillus *P* is always present in very

TABLE VI(*g*).

1

BERHAMPORE RIVER.—9-7-11.

			Glucose.		Lactose.	
			+	−	+	−
1	..	20	1	*Nil*	1	*Nil*
2	..	10	2	*Nil*	2	*Nil*
3	..	5	3	*Nil*	3	*Nil*
3	..	1	3	*Nil*	3	*Nil*
3	..	·1	3	*Nil*	3	*Nil*
3	..	·05	3	*Nil*	3	*Nil*
3	..	·03	3	*Nil*	3	*Nil*
3	..	·01	3	*Nil*	3	*Nil*
3	..	·005	2	1	1	2
3	..	·003	1	2	1	2
3	..	·001	*Nil*	3	*Nil*	3
			·005		·01	

	Sacchar.	Dulcite.	Adonite.	Inulin.	V. & P.	Indol.	Motility.	Name or Number.
1	+	+	−	−	+	−	−	75
2	−	−	−	−	−	+	−	Vesiculosus.
3	−	−	−	−	−	+	−	Do.
4	+	−	+	−	+	−	−	L. ærogenes
5	+	+	−	−	−	−	−	?
6	−	+	−	−	−	+	−	Schäfferi.
7	+	+	+	−	+	−	−	67
8	+	−	+	−	+	−	−	L. ærogenes.
9	−	−	−	−	−	+	−	Vesiculosus.
10	−	−	−	−	−	+	−	Do.

2

BERHAMPORE RIVER.—17-10-11.

			Glucose.		Lactose.	
			+	−	+	−
1	..	20	1	*Nil*	1	*Nil*
2	..	10	2	*Nil*	2	*Nil*
3	..	5	3	*Nil*	3	*Nil*
3	..	1	3	*Nil*	3	*Nil*
3	..	·1	3	*Nil*	3	*Nil*
3	..	·05	3	*Nil*	2	1
3	..	·03	3	*Nil*	1	2
3	..	·01	*Nil*	3	*Nil*	3
3	..	·005	*Nil*	3	*Nil*	3
3	..	·003	*Nil*	3	*Nil*	3
3	..	·001	*Nil*	3	*Nil*	3
			·03		·05	

	Sacchar.	Dulcite.	Adonite.	Inulin.	V. & P.	Indol.	Motility.	Name or Number.
1	−	−	−	−	−	+	−	Vesiculosus.
2	−	−	−	−	−	+	−	Do.
3	+	−	−	−	−	+	−	Coscoroba.
4	−	−	−	−	−	+	−	Vesiculosus.
5	−	−	−	−	−	+	−	Do.
6	−	−	−	−	−	+	−	Do.
7	+	−	+	−	+	−	−	L. ærogenes.
8	−	−	−	−	−	+	−	Vesiculosus.
9	−	+	−	−	−	+	−	Schäfferi.
10	−	+	−	−	−	+	−	Neapolitanus.

3

NAIHATI.—13-8-11.

		Glucose. +	Glucose. −	Lactose. +	Lactose. −
1	.. 20	1	*Nil*	1	*Nil*
2	.. 10	2	*Nil*	2	*Nil*
3	.. 5	3	*Nil*	3	*Nil*
3	.. 1	3	*Nil*	3	*Nil*
3	.. ·1	3	*Nil*	3	*Nil*
3	.. ·05	3	*Nil*	3	*Nil*
3	.. ·03	3	*Nil*	3	*Nil*
3	.. ·01	3	*Nil*	2	1
3	.. ·005	3	*Nil*	2	1
3	.. ·003	2	1	*Nil*	3
3	.. ·001	2	1	*Nil*	3
		·001		·005	

	Sacchar.	Dulcite.	Adonite.	Inulin.	V. & P.	Indol.	Motility.	Name or Number.
1	+	+	−	−	−	+	−	Neapolitanus.
2	+	+	−	−	−	+	−	Do.
3	+	+	−	−	−	+	−	Do.
4	+	−	−	−	+	−	−	Cloacæ.
5	+	−	−	−	+	−	−	Do.
6	+	+	−	−	−	+	−	Neapolitanus.
7	−	−	−	−	−	+	−	Vesiculosus.
8	−	−	−	−	−	+	−	Do.
9	−	+	−	−	−	+	−	Schäfferi.
10	−	−	+	−	−	+	−	A. lactici.

4

NAIHATI.—13-10-11.

		Glucose. +	Glucose. −	Lactose. +	Lactose. −
1	.. 20	1	*Nil*	1	*Nil*
2	.. 10	2	*Nil*	2	*Nil*
3	.. 5	3	*Nil*	3	*Nil*
3	.. 1	3	*Nil*	3	*Nil*
3	.. ·1	3	*Nil*	3	*Nil*
3	.. ·05	3	*Nil*	3	*Nil*
3	.. ·03	3	*Nil*	3	*Nil*
3	.. ·01	3	*Nil*	3	*Nil*
3	.. ·005	3	*Nil*	3	*Nil*
3	.. ·003	3	*Nil*	1	2
3	.. ·001	1	2	1	2
		·003		·005	

	Sacchar.	Dulcite.	Adonite.	Inulin.	V. & P.	Indol.	Motility.	Name or Number.
1	+	−	+	−	+	−	−	L. ærogenes.
2	+	−	−	−	−	+	−	Coscoroba.
3	−	−	−	−	−	+	−	Vesiculosus.
4	+	−	−	−	−	+	−	Coscoroba.
5	+	+	+	−	+	−	−	No. 67
6	+	−	+	−	+	−	−	L. ærogenes.
7	−	−	+	−	−	+	−	Vesiculosus.
8	+	−	+	−	−	+	−	Coscoroba.
9	+	−	+	−	−	+	−	Do.
10	+	+	+	−	−	+	−	Neapolitanus.

5

BUXAR RIVER.—29-6-11.

		Glucose. +	Glucose. −	Lactose. +	Lactose. −
1	.. 20	1	*Nil*	1	*Nil*
2	.. 10	2	*Nil*	2	*Nil*
3	.. 5	3	*Nil*	3	*Nil*
3	.. 1	3	*Nil*	3	*Nil*
3	.. .1	3	*Nil*	3	*Nil*
3	.. ·05	3	*Nil*	3	*Nil*
3	.. ·03	3	*Nil*	3	*Nil*
3	.. ·01	3	*Nil*	3	*Nil*
3	.. ·005	3	*Nil*	2	1
3	.. ·003	*Nil*	3	*Nil*	3
3	.. ·001	*Nil*	3	*Nil*	3
		·005		·005	

	Sacchar.	Dulcite.	Adonite.	Inulin.	V. & P.	Indol.	Motility.	Name or Number.
1	+	−	+	−	−	−	−	Gasiformans.
2	−	+	−	−	−	+	−	Schäfferi.
3	+	−	+	−	−	+	−	Gasiformans.
4	−	+	−	−	−	+	−	Schäfferi.
5	−	+	−	−	−	+	−	Do.
6	+	−	+	−	−	−	−	Gasiformans.
7	+	−	−	−	−	+	−	Coscoroba.
8	+	−	+	−	+	−	−	L. ærogenes.
9	+	+	−	−	−	+	−	Nepolitanus.
10	−	−	+	−	−	+	−	Acidi lactici.

6

BUXAR RIVER.—24-8-11.

			Glucose. +	Glucose. –	Lactose. +	Lactose. –
1	..	20	1	*Nil*	1	*Nil*
2	..	10	2	*Nil*	2	*Nil*
3	..	5	3	*Nil*	3	*Nil*
3	..	1	3	*Nil*	3	*Nil*
3	..	.1	3	*Nil*	3	*Nil*
3	..	.05	3	*Nil*	3	*Nil*
3	..	.03	3	*Nil*	3	*Nil*
3	..	.01	3	*Nil*	3	*Nil*
3	..	.005	3	*Nil*	3	*Nil*
3	..	.003	1	2	2	1
3	..	.001	*Nil*	3	*Nil*	3
			·005		·003	

	Sacchar.	Dulcite.	Adonite.	Inulin.	V. & P.	Indol.	Motility.	Name or Number.
1	+	–	+	–	+	–	–	L. ærogenes.
2	+	–	+	–	+	–	–	Do.
3	+	–	+	–	–	–	–	Gasiformans.
4	+	–	+	–	–	–	–	Do.
5	+	–	+	–	+	–	–	L. ærogenes.
6	+	–	+	–	+	–	–	Do.
7	+	–	+	–	+	–	–	Do.
8	+	+	+	–	+	–	–	67
9	–	+	–	–	–	+	+	Coli. Com.
10	+	–	+	–	–	–	–	Gasiformans.

7

BUXAR SETTLED.—48 hrs.—24-8-11.

			Glucose. +	Glucose. –	Lactose. +	Lactose. –
1	..	20	1	*Nil*	1	*Nil*
2	..	10	2	*Nil*	2	*Nil*
3	..	5	3	*Nil*	3	*Nil*
3	..	1	3	*Nil*	3	*Nil*
3	..	.1	3	*Nil*	3	*Nil*
3	..	.05	*Nil*	3	*Nil*	3
3	..	.03	*Nil*	3	*Nil*	3
3	..	.01	*Nil*	3	*Nil*	3
3	..	.005	*Nil*	3	*Nil*	3
3	..	.003	*Nil*	3	*Nil*	3
3	..	.001	*Nil*	3	*Nil*	3
			·1		·1	

	Sacchar.	Dulcite.	Adonite.	Inulin.	V. & P.	Indol.	Motility.	Name or Number.
1	–	–	–	–	–	+	–	Grunthal.
2	–	–	–	–	–	+	–	Vesiculosus.
3	+	+	+	–	–	+	–	Neapolitanus.
4	–	–	–	–	–	+	–	Grunthal.
5	–	–	–	–	–	+	–	Vesiculosus.
6	–	+	–	–	–	+	–	Schäfferi.
7	–	–	–	–	+	+	–	Vesiculosus.
8	–	–	–	+	–	+	–	Do.
9	+	–	+	–	–	–	–	L. ærogenes.
10	+	–	+	–	–	–	–	Do.

8

HUGHLY (Howrah Bridge).—16-12-11.

			Glucose. +	Glucose. –	Lactose. +	Lactose. –
1	..	20	1	*Nil*	1	*Nil*
2	..	10	2	*Nil*	2	*Nil*
3	..	5	3	*Nil*	3	*Nil*
3	..	1	3	*Nil*	3	*Nil*
3	..	.1	3	*Nil*	3	*Nil*
3	..	.05	*Nil*	3	1	2
3	..	.03	*Nil*	3	1	2
3	..	.01	1	2	*Nil*	3
3	..	.005	*Nil*	3	*Nil*	3
3	..	.003	*Nil*	3	*Nil*	3
3	..	.001	*Nil*	3	*Nil*	3
				·1		·1

	Sacchar.	Dulcite.	Adonite.	Inulin.	V. & P.	Indol.	Motility.	Name or Number.
1	–	+	–	–	–	+	–	Schäfferi.
2	+	–	+	–	+	–	–	L. ærogenes.
3	+	–	+	–	+	–	–	Do.
4	+	–	–	–	–	+	–	Coscoroba.
5	–	–	–	–	–	+	–	Vesiculosus.
6	+	–	–	–	–	+	–	Coscoroba.
7	+	–	–	–	+	+	–	Do.
8	+	–	+	–	+	–	–	L. ærogenes.
9	+	–	–	–	–	–	–	No. 111.
10	–	–	–	–	–	+	–	Vesiculosus.

Streptococci present in 1 cc.

9

ARRAH RIVER.—22-7-11.

			Glucose.		Lactose.	
			+	−	+	−
1	..	20	1	*Nil*	1	*Nil*
2	..	10	2	*Nil*	2	*Nil*
3	..	5	3	*Nil*	3	*Nil*
3	..	1	3	*Nil*	3	*Nil*
3	..	·1	3	*Nil*	2	1
3	..	·05	3	*Nil*	3	*Nil*
3	..	·03	3	*Nil*	*Nil*	3
3	..	·01	1	2	*Nil*	3
3	..	·005	*Nil*	3	*Nil*	3
3	..	·003	*Nil*	3	*Nil*	3
3	..	·001	*Nil*	3	*Nil*	3
				·03		·05

	Sacchar.	Dulcite.	Adonite.	Inulin.	V. & P.	Indol.	Motility.	Name or Number.
1	+	−	+	−	−	−	−	Gasiformans.
2	+	−	+	−	+	−	−	L. ærogenes.
3	−	+	−	−	−	+	−	Schäfferi.
4	−	+	−	−	−	+	−	Do.
5	+	+	+	−	+	−	−	No. 67
6	+	+	+	−	+	−	−	Do.
7	+	+	+	−	+	−	−	Do.
8	−	+	−	−	−	+	−	Schäfferi.
9	+	+	+	−	−	−	−	Pneumoniæ.
10	+	−	+	−	+	−	−	L. ærogenes.

10

BHAGHALPUR.—28-7-11.

			Glucose.		Lactose.	
			+	−	+	−
1	..	20	1	*Nil*	1	*Nil*
2	..	10	2	*Nil*	2	*Nil*
3	..	5	3	*Nil*	3	*Nil*
3	..	1	3	*Nil*	3	*Nil*
3	..	·1	3	*Nil*	3	*Nil*
3	..	·05	3	*Nil*	3	*Nil*
3	..	·03	3	*Nil*	3	*Nil*
3	..	·01	*Nil*	3	1	2
3	..	·005	*Nil*	3	*Nil*	3
3	..	·003	*Nil*	3	*Nil*	3
3	..	·001	*Nil*	3	*Nil*	3
				·03		·03

	Sacchar.	Dulcite.	Adonite.	Inulin.	V. & P.	Indol.	Motility.	Name or Number.
1	+	+	−	−	−	+	−	Neapolitanus.
2	+	+	−	−	−	+	−	Do.
3	−	+	−	−	−	+	−	Schäfferi.
4	+	+	−	−	−	+	−	Neapolitanus.
5	+	−	−	−	−	+	−	Coscoroba.
6	−	+	−	−	−	+	−	Schäfferi.
7	−	+	−	−	−	+	−	Do.
8	+	+	−	−	−	+	−	Neapolitanus.
9	+	+	−	−	−	+	−	Do.
10	−	+	−	−	−	+	−	Schäfferi.

11.

BHAGHALPUR RIVER.—28-7-11.

			Glucose.		Lactose.	
			+	−	+	−
1	..	20	1	*Nil*	1	*Nil*
2	..	10	2	*Nil*	2	*Nil*
3	..	5	3	*Nil*	3	*Nil*
3	..	1	3	*Nil*	3	*Nil*
3	..	·1	3	*Nil*	3	*Nil*
3	..	·05	3	*Nil*	3	*Nil*
3	..	·03	3	*Nil*	1	2
3	..	·01	*Nil*	3	*Nil*	3
3	..	·005	*Nil*	3	*Nil*	3
3	..	·003	*Nil*	3	*Nil*	3
3	..	·001	*Nil*	3	*Nil*	3
				·03		·05

	Sacchar.	Dulcite.	Adonite.	Inulin.	V. & P.	Indol.	Motility.	Name or Number.
1	−	+	−	−	−	+	−	Schäfferi.
2	+	+	−	−	−	+	−	Neapolitanus.
3	+	+	−	−	−	+	−	Do.
4	+	+	−	−	−	+	−	Do.
5	−	+	−	−	−	+	−	Schäfferi.
6	−	+	−	−	−	+	−	Do.
7	−	+	−	−	−	+	−	Do.
8	−	+	−	−	−	+	−	Do.
9	+	+	−	−	−	+	−	Neapolitanus.
10	−	+	−	−	−	+	−	Schäfferi.

12.

BURDWAN RIVER.—16-8-11.

			Glucose. +	Glucose. −	Lactose. +	Lactose. −
1	..	20	1	*Nil*	1	*Nil*
2	..	10	2	*Nil*	2	*Nil*
3	..	5	3	*Nil*	3	*Nil*
3	..	1	3	*Nil*	3	*Nil*
3	..	·1	3	*Nil*	3	*Nil*
3	..	·05	3	*Nil*	2	1
3	..	·03	3	*Nil*	1	2
3	..	·01	3	*Nil*	*Nil*	3
3	..	·005	1	2	*Nil*	3
3	..	·003	*Nil*	3	*Nil*	3
3	..	·001	*Nil*	3	*Nil*	3
				·01		·05

	Sacchar.	Dulcite.	Adonite.	Inulin.	V. & P.	Indol.	Motility.	Name or Number.
1	−	−	+	−	−	+	−	A. lactici.
2	+	−	−	−	−	+	−	Coscoroba.
3	−	+	−	−	−	+	+	Coli. Com.
4	+	+	−	−	−	+	−	Neapolitanus.
5	−	+	−	−	−	+	−	Schäfferi.
6	+	−	+	−	−	+	−	....
7	+	−	−	−	−	+	−	Coscoroba.
8	−	+	−	−	−	+	−	Schäfferi.
9	+	−	+	−	−	+	−	Gasiformans.
10	+	−	+	−	+	−	−	L. ærogenes.

Observe in all these samples the closeness of the acid and gas line in glucose and lactose—the number of varieties in 10 colonies—the presence of Coli Communis or Schäfferi—and the general appearance of the mixture of bacilli.

large numbers, *vide* analyses 13-17, Table VI(*h*). The samples taken during this period show that the rivers present all the characteristics of ordinary dry weather samples.

TABLE VI (*h*).

ALL THESE ARE DRY WEATHER SAMPLES. OBSERVE THE RELATIVE FEWNESS OF BACILLI—THE FEW VARIETIES IN 10 COLONIES—THE ENTIRE ABSENCE OF COLI COMMUNIS.

13

NAIHATI.—9-11-11.

			Glucose. +	Glucose. −	Lactose. +	Lactose. −
1	..	20	1	*Nil*	1	*Nil*
2	..	10	2	*Nil*	2	*Nil*
3	..	5	3	*Nil*	3	*Nil*
3	..	1	3	*Nil*	3	*Nil*
3	..	·1	3	*Nil*	3	*Nil*
3	..	·05	3	*Nil*	1	2
3	..	·03	3	*Nil*	1	2
3	..	·01	3	*Nil*	*Nil*	3
3	..	·005	3	*Nil*	*Nil*	3
3	..	·003	1	2	*Nil*	3
3	..	·001	1	2	*Nil*	3
				·005		·1

	Sacchar.	Dulcite.	Adonite.	Inulin.	V. & P.	Indol.	Motility.	Name or Number.
1	+	−	+	−	+	−	−	L. ærogenes.
2	+	−	+	−	+	−	−	Do.
3	+	−	+	−	+	−	−	Do.
4	+	−	+	−	+	−	−	Do.
5	+	−	+	−	+	−	−	Do.
6	+	−	+	−	+	−	−	Do.
7	+	−	+	−	+	−	−	Do.
8	+	−	+	−	+	−	−	Do.
9	+	−	+	−	+	−	−	Do.
10	+	−	+	−	+	−	−	Do.

14

ARRAH RIVER.—13-4-11.

			Glucose. +	Glucose. −	Lactose. +	Lactose. −
1	..	20	1	*Nil*	1	*Nil*
2	..	10	2	*Nil*	2	*Nil*
3	..	5	3	*Nil*	2	1
3	..	1	3	*Nil*	*Nil*	3
3	..	·1	3	*Nil*	*Nil*	3
3	..	·05	..	..	..	..
3	..	·03	..	..	..	..
3	..	·01	*Nil*	3	*Nil*	3
3	..	·005	..	..	..	..
3	..	·003	..	..	..	..
3	..	·001	*Nil*	3	*Nil*	3
				·1		5

	Sacchar.	Dulcite.	Adonite.	Inulin.	V. & P.	Indol.	Motility.	Name or Number.
1	−	−	−	−	−	+	−	Vesiculosus.
2	−	−	−	−	−	+	−	Do.
3	−	−	+	−	−	+	−	A. lactici.
4	−	−	−	−	−	+	−	Vesiculosus.
5	−	−	+	−	−	+	−	A. lactici.
6	−	−	+	−	−	+	−	Do.
7	−	−	−	−	−	+	−	Vesiculosus.
8	−	−	−	−	−	+	−	Do.
9	−	−	+	−	−	+	−	A. lactici.
10	−	−	−	−	−	+	−	Vesiculosus.

15

MONGHYR RIVER.—26-5-11.

			Glucose. +	Glucose. −	Lactose. +	Lactose. −
1	..	20	1	*Nil*	1	*Nil*
2	..	10	2	*Nil*	2	*Nil*
3	..	5	3	*Nil*	*Nil*	3
3	..	1	3	*Nil*	*Nil*	3
3	..	·1	*Nil*	3	*Nil*	3
3	..	·05	..	..	..	..
3	..	·03	..	..	..	..
3	..	·01	*Nil*	3	*Nil*	3
3	..	·005	..	..	..	..
3	..	·003	..	..	..	..
3	..	·001	..	..	..	..
				1		10

	Sacchar.	Dulcite.	Adonite.	Inulin.	V. & P.	Indol.	Motility.	Name or Number.
1	+	−	−	−	−	+	−	Coscoroba.
2	+	−	+	−	+	−	−	L. ærogenes.
3	+	−	−	−	−	−	−	Coscoroba.
4	+	−	−	−	−	+	−	Do.
5	+	−	−	−	−	+	−	Do.
6	+	−	−	−	−	+	−	Do.
7	+	−	−	−	−	+	−	Do.
8	+	−	−	−	−	+	−	Do.
9	+	−	−	−	−	+	−	Do.
10	+	−	−	−	−	+	−	Do.

16

DALTANGUNJ RIVER.—10-12-11.

			Glucose. +	Glucose. −	Lactose. +	Lactose. −
1	..	20	1	*Nil*	1	*Nil*
2	..	10	2	*Nil*	2	*Nil*
3	..	5	3	*Nil*	3	*Nil*
3	..	1	3	*Nil*	3	*Nil*
3	..	·1	3	*Nil*	1	2
3	..	·05	3	*Nil*	1	2
3	..	·03	3	*Nil*	*Nil*	3
3	..	·01	*Nil*	3	*Nil*	3
3	..	·005	1	2	*Nil*	3
3	..	·003	*Nil*	3	*Nil*	3
3	..	·001	*Nil*	3	*Nil*	3
			.03			1

	Sacchar.	Dulcite.	Adonite.	Inulin.	V. & P.	Indol.	Motility.	Name or Number.
1	−	−	−	−	−	+	−	Vesiculosus.
2	−	−	−	−	−	+	−	Do.
3	−	−	−	−	−	+	−	Do.
4	−	−	−	−	−	+	−	Do.
5	+	+	−	−	−	+	−	Neapolitanus.
6	+	+	−	−	−	+	−	Do.
7	−	−	−	−	−	+	−	Vesiculosus.
8	−	−	−	−	−	+	−	Do.
9	−	−	−	−	−	+	−	Do.
10	−	−	−	−	−	+	−	Do.

17

BHAGHALPUR.—25-11-11.

			Glucose.		Lactose.	
			+	−	+	−
1	..	20	1	*Nil*	1	*Nil*
2	..	10	2	*Nil*	2	*Nil*
3	..	5	3	*Nil*	3	*Nil*
3	..	1	3	*Nil*	2	1
3	..	·1	3	*Nil*	*Nil*	3
3	..	·05	1	2	*Nil*	3
3	..	·03	1	2	*Nil*	3
3	..	·01	1	2	*Nil*	3
3	..	·005	*Nil*	3	*Nil*	3
3	..	·003	*Nil*	3	*Nil*	3
3	..	·001	*Nil*	3	*Nil*	3
				·1		1

	Sacchar.	Dulcite.	Adonite.	Inulin.	V. & P.	Indol.	Motility.	Name or Number.
1	−	−	−	−	−	+	−	Vesiculosus.
2	+	−	−	−	−	+	−	Coscoroba.
3	+	−	−	−	−	+	−	Do.
4	−	−	−	−	−	−	−	Mutabilis.
5	+	−	−	−	−	+	−	Coscoroba.
6	+	−	−	−	−	+	−	Do.
7	+	−	−	−	+	−	−	Cloacæ.
8	+	−	+	−	+	−	−	L. ærogenes.
9	+	−	+	−	+	−	−	Do.
10	+	−	−	−	−	+	−	Coscoroba.

Another most interesting fact is also shown by our work, *viz.*, that out of 35 samples of river water taken at Howrah Bridge, where we know there is gross pollution from Calcutta and Howrah, not more than about 5% of all samples taken in wet and dry weather show any appreciable widening of the acid and gas line in the two media.

Of course, it is apparent that work on rivers can never give as definite results as on lakes, because the whole way along both banks more or less pollution is being added. It is possible to select a lake for research work where we know that actual pollution is only added when rain is washing it from the catchment area into the lake.

When one considers the extent of these investigations, coupled with the results obtained by others already quoted, there can be no reasonable doubt that a very vital change takes place in the arrangement of organisms which are present in fresh fæces after it has passed into water. The extreme rarity of the group glucose + lactose — has already been mentioned, and yet a few days after fæcal material has passed into either rivers or lakes, these organisms greatly predominate. Again, the results of the work mentioned above demonstrate the fact *that the more recent the pollution, the closer is the acid and gas line in the two media to one another.* This latter statement is of great importance, and we shall show later on that it can be of greater service to the water analysts.

Let us now deal with the actual explanation of these facts. From the above figures one would be inclined to come to the conclusion that the class of organisms glucose + lactose – was, as a whole, more resistant to natural adverse influences; or to put it in another way, they were in more congenial environment in water than the class glucose + lactose +. This explanation obviously satisfies the conditions commonly met with in nature, so we promptly started to demonstrate the truth of the statement by experiments. In order to do this a mixture of fæces and water was made, the gross particles removed by filtering it through cotton-wool, the filtrate placed in a flat photographic dish, and put out in the sun. If our contention was correct, the probabilities were that all the lactose fermenting organisms should have disappeared leaving the glucose + lactose – behind. A large series of these experiments were carried out with somewhat astonishing results. It was clearly shown that, at any rate as a class glucose + lactose – organisms were not any more resistant to the action of sunlight than were the glucose + lactose +, for in 9 cases out of 10 both classes of organisms died out with apparently almost equal rapidity. In some of the experiments there was a slight indication that the lactose fermenting organisms disappear rather more rapidly than the others, but in an experiment of this kind both classes were, so to speak, so much at the mercy of the sunlight, that we entirely failed to reproduce the conditions that exist in nature. The sunlight experiments, however, demonstrated one point with remarkable clearness, namely, that when the mixture was made the bacillus *P* was so rare that it could practically never be isolated, yet at the end of the experiment *in every case* just before the mixture became sterile, only this organism could be found. It was this remarkable fact that gave us the clue to the true explanation of what occurs in nature. Further work on samples of natural water was commenced, and from a very careful and lengthy investigation of the surviving members of the class glucose + lactose – obtained from rivers, naturally polluted lakes, artificially polluted tanks, absolutely uniform results were obtained, *viz., that the only organism of this class that could be isolated from samples taken after some exposure to the sun's rays was bacillus P.*

This is a very important conclusion, because it shows the utter futility of making such a statement as "*all* organisms giving certain fermentation reaction indicate objectionable pollution." When we come to deal with the action of sunlight on the lactose fermenting group, we shall show that the same thing applies, for there is a very great difference between the way in which the individual members of a group behave when they are passed into water. It is not maintained that no other member of the group glucose + lactose — is likewise resistant, or may behave in exactly the same as bacillus *P*, but we have not found this to be the case up to now.

The importance of this result is considerable, for it gives the correct explanation of the phenomena already recorded. It appears to be as follows :—The coincidence of the acid and gas line in the two broths in recent pollution is due to the great rarity of the class glucose + lactose — as a whole, and bacillus *P* in particular, in fresh fæces. The gradual fall in the number of both is due to the dying off of hundreds of both classes ; the gradual widening of the line is due to the multiplication of one individual organism, *viz.*, bacillus *P*. This widening we shall see later on may be masked by a precisely similar multiplication of a certain member of the glucose + lactose + class.

From the great ubiquity of bacillus *P* it must be assumed that the organism is in very congenial surroundings in almost any water, for it is found in clear and dirty alike, in lakes nearly dry and in good river waters ; the only place where it is looked for in vain is in water recently contaminated with sewage.

In the light of this conclusion it is obvious that a study of these natural phenomena may be of great use to the water analyst, for here is a means of ascertaining whether the true fæcal arrangement has had time to become seriously disturbed or not. It takes time both for the bacillus *P* to grow, and for the lactose fermenters to die off, but broadly speaking, it is true that the wider the divergence in the acid and gas line in glucose lactose broth, the older is the pollution. Further, it is astonishing how really sensitive for *surface waters* this test is, and what a comparatively small volume of recent pollution brings the acid and gas line in the two media together. The standing example of this is the condition of the water at the Howrah Bridge. We have already stated that the pollution is relatively small in amount, though gross in character, yet divergence in the acid and gas line is rare at any time of year. Of course it is not maintained that simply finding the acid and gas line in the two broths will tell the analyst everything he wants to know about a sample, but it will undoubtedly give much valuable information.

The next question we have to decide is :—What is the best laboratory method of estimating the relative numbers of the two classes of organisms ?

In the course of our investigations we have always observed that, during the summer months, even when using a lactose broth and lactose solid medium we get a great preponderance of *white* colonies on the agar. (Investigation shows that 95 per cent. of all these organisms are bacillus *P*.) But if a glucose broth is used for the first step, practically no red colonies can be isolated at this time. This being so it was thought that it might be possible to get a rough indication of the relative number of these two groups of organisms (lactose + glucose +, and lactose — glucose +) by counting the white and red colonies that grow on the agar medium. A large number of figures have been compiled during months of research and we find that this method is of very little value for the following reasons :—

It was discovered that the relation between the number of white and red colonies in any given sample depended almost entirely upon *which tube of the series was used for making the plates*. Thus with a sample of a well stored water, supposing in the lactose broth series the 20, the 10, the 5, the 1 and the ·1 c.c. had all given acid and gas, if plates were made from the 20 c.c. red colonies would predominate, probably a few white colonies would be obtained ; if from the 1 or ·1 c.c. (the last one of the series which gave acid and gas) practically no red colonies at all would be found on the plate ;

if the 5 or 10 c.c. were used the red colonies and the white would be fairly equally mixed. The explanation of this phenomenon is not so simple, but it is probably dependent on the fact that lactose fermenters, when they are sufficiently numerous, overgrow the glucose fermenters. At any rate whatever the explanation may be there is absolutely no doubt of the truth of the above finding, and we may consequently conclude that the counting of red and white colonies on agar gives apparently no indication as to the relative frequency of the two classes of organisms present in the water itself.

It may be contended that in the light of this knowledge Table No. VI(*b*) is worthless. As a matter of fact that is not quite true, because during this investigation the last tube but one that gave the reaction was always taken for plating out colonies. Consequently, although the actual percentage relations between the white and red colonies in any given sample may be of no particular value, the variation due to different times of the year, and between the different samples is of some value because whatever the error may be, it is the same for each sample. We do not, however, place great importance on the Table VI(*b*), because the method followed has been subsequently shown to contain these fallacies.

From a very long and careful study we have come to the conclusion that *the only possible way of estimating the relative number of glucose + lactose + and glucose + lactose — is by putting identical quantities and dilutions of the sample into both broths and observing where the acid and gas line comes in each.* There is no doubt whatever that this procedure is extraordinarily accurate; if 3 or 5 tubes of each dilution are used, a very full knowledge of the condition of the water is obtained. Of course, it is necessary to put in various steps between ·1 and ·01.

The absolute reliability of this method has been shown by the following accident. In a few of our sunlight experiments a sample of fæces was used for making the emulsion which contained no glucose + lactose — organisms at all. *In every estimation made from the start to finish the glucose and lactose line coincided exactly.*

Further, if half a dozen separate estimations are made in both broths with the same sample, it is most unusual to obtain any difference in result between any of the six. We have never discovered any fallacies in this method of estimating the number of any class of organisms.

One question is still left to be discussed, *viz.*, in most samples, is it worth while to go to the trouble of finding out the acid and gas line in glucose and lactose broth ? The answer to this appears to be that in cases where very accurate knowledge of the condition of the water at any particular time is desired, it is worth carrying out this test, troublesome though it is. For instance (this case actually occurred), in a raw river water a sample taken at the intake of a town supply showed that the acid and gas line in both broths was very close together, as compared with a sample from the middle of the river. Yet the number of glucose fermenters was about the same in the two samples. Inspection showed that the bank just above the intake was badly fouled by mill coolies. Again, if outside pollution finds its way into a filtered drinking water containing a large number of glucose fermenters, but few lactose fermenters, a rapid drawing together of the lines would be very strong evidence of pollution, though the increase in total number of glucose fermenters might be overlooked. Of course in all cases other evidence of pollution would probably be available, if thorough investigation was made.

In all cases of raw water where there is no doubt as to the fact that pollution is present, it is often particularly desirable to know whether the pollution is recent or old standing ; or in other words, does the pollution still exist in the true fæcal form or has it been much altered by natural forces ? In all such instances, it would be well worth while to put the sample through both glucose and lactose broth. The following conclusions are justified from the above results :

(*i*) that when dealing with natural waters the acid and gas line in glucose broth is usually higher than in lactose.

(*ii*) that the relation between the two classes of organisms varies at different times of the year in natural samples of surface water. That the divergence is greater in summer than winter;

(*iii*) that in lake waters the divergence is more marked than in river waters;

(*iv*) that experiment and a careful study of rivers and lakes shows that the high acid and gas line in glucose is due to the presence of bacillus *P* which multiplies in water;

(*v*) speaking broadly the more recent the pollution is, the nearer together is the acid and gas line in glucose and lactose broth, the older it is, the greater the divergence;

(*vi*) that the best way of estimating the numbers of the glucose + lactose – and glucose + lactose + fæcal organisms is by using the same quantity of the water in the two broth media.

CHAPTER VII.

THE ACTION OF DIRECT SUNLIGHT ON THE COMMON FÆCAL ORGANISMS.

WE now proceed with a study of the effect of natural influences on the common lactose fermenting fæcal organisms. These, as we have seen, form by far the greater proportion of what may be termed the "coliform" organisms in fæces, and are, on account of their numerical importance, the variety that should first be searched for in making study of the pollution of water. The results quoted in Chapter V show that there is really no doubt that the common intestinal organism is a true lactose fermenter, so in dealing with a sample of water, unless a very full analysis is done, a study of the lactose fermenters will probably give the analyst most information. We entirely agree with MacConkey's statement that a lactose broth is preferable to a glucose broth for ordinary work.

From 4 years study of ordinary lactose fermenting organisms, several very interesting points have been discovered; the most important of these has been obtained by what we call sunlight experiments. A reference to this work has already been made in the earlier chapters, but it is now necessary to describe the method in use more fully. A mixture of a known quantity of fæces (usually one gramme per litre) is made in water, the grosser particles are filtered off with cotton-wool and the emulsion is placed in a flat dish in the sun, sometimes adding a layer of sterile sand at the bottom of the dish in order to make the experiment somewhat more natural. Samples of this emulsion are taken and carefully analysed at various intervals in order to see what alterations in the bacterial flora have taken place. Upwards of 50 of these experiments have been carried out during the course of our work, and we find that within broad lines certain varieties of lactose fermenting organisms disappear with much greater rapidity than do the others; that is to say, we find that there are some varieties which are very susceptible to the action of sunlight, there are some which appear to be very resistant, and there is a third group which may be called intermediate between the two extremes.

It should be explained that the reasons for working with emulsions of fresh human and animal fæces were that from a series of experiments carried out on laboratory cultures, we find that these latter behave in an entirely different way from the organisms obtained direct from the intestinal tracts of man and animals. It soon became apparent that any conclusions which applied to fresh fæces did not necessarily apply to laboratory cultures of the same organism. This, of course, may be brought forward as an objection to this form of experiment.

Let us now give the details of the experiments carried out. In the first instance we attempted to show that by placing a mixture, such as the one described above, in the sun and protecting it from outside contamination that bacilli present actually decrease in numbers fairly rapidly. This was not by any means a difficult point to prove, and all our experiments showed that this disappearance was fairly consistent and rapid. One experiment, Table No. VII (*a*), is given as an example for the rest, there being no necessity to describe in detail every separate experiment.

Having established the fact that fæcal organisms placed in water in the sun do disappear, we went a step further in order to ascertain whether there was any difference in the rapidity with which the separate species of organisms vanish. About 50 such experiments on this line have been carried out, 10 of the most typical of these have been selected and given in detail below. Selecting experiments may be open to objection, but it is needless to give details of all those carried out, many of them are not of much value because susceptible and resistant organisms were not present in the original emulsion.

Before giving the actual results of these experiments it is necessary to make a few comments.

In all bacteriological work the method chosen has its own limitations; that followed by us is no exception to the rule. Although 10 colonies, all of which are carefully sub-

TABLE VII(*a*).

	.001 cc.	.01 cc.	.1 cc.	1 cc.	5 cc.	10 cc.	20 cc.	REMARKS.
Experiment No. 11.								
17th Jan. 1909, before exposure, noon.	+ + +	+ + +	+ + +					
17th Jan. 1909, 2 P.M. ..	+ + +	+ + +	+ + +					
,, 5 P.M. ..	— + +	+ + +	+ + +					
18th Jan. 1909, 2 P.M. ..	— — +	+ + +	+ + +					
,, 3 P.M. ..	+ + +	— — +	+ + +					
,, 5 P.M. ..	— — —	+ + +	+ + +					
19th Jan. 1909, 1 P.M. ..	— — —	— — +	+ + +					
,, 3 P.M. ..	— — —	— — +	— + +	+ + +				
,, 5 P.M. ..	— + +	+ + +	+ + +	+ + +				Sediment taken.
20th Jan. 1909, 2 P.M. ..	— — —	— — —	— — —	+ + +				
,, 5 P.M. ..	— — —	— — —	— — —	— — —	— — —			
21st Jan. 1909, 2 P.M. ..			— — +	— — +	— — —			
,, 5 P.M. ..			— — +	— — +	— — —	— +	+	
22nd Jan. 1909, 12 noon ..				— — +	— — +			
,, 5 P.M. ..				— — —	+ + +	+ +	+	
23rd Jan. 1909, 5 P.M. ..				— — —	— — +	— —		
24th Jan. 1909, 5 P.M. ..				— — —	— — —	— —	—	
25th Jan. 1909, 5 P.M. ..					— — —	— —	—	
26th Jan. 1909, 5 P.M. ..								

cultured, gives a fair estimate of the organisms present in any sample, the actual number of organisms present is an important factor. In the emulsion of fæces used by us, at the beginning of these experiments there were from 1,000 to 10,000 fæcal organisms per c.c. Obviously 10 colonies selected from the organisms in 20 c.c. of this emulsion, can only give the organisms which preponderate numerically, the chances of isolating rare organisms being very small. But when the action of the sun has reduced the number of organisms from 10,000 per c.c. to 10—100 per c.c., then it is reasonably certain that our methods of analysis give results which actually represent the flora of the emulsion at the time.

On the other hand, when the organisms have been reduced to one in 50, or one in 100 c.c., another error has to be reckoned with ; in the unfiltered emulsions it is common to get small particles of fæcal matter, surrounded with an envelope of mucus, which may contain 3 or 4 different organisms that have, in this way, escaped the action of the rays of the sun. Examples of this accident have occurred in our experiments. Applying these remarks to the sunlight experiments, it is obvious that the early analyses on each case are approximate only, whilst the later ones (in so far as they are free from the second of these errors) are of very considerable value, and give a more correct estimate of the organisms present.

Let us now say something about the various experiments commencing with No. 1. Unfortunately we have never again succeeded in getting such an instructive sample of human fæces or cowdung as this one proved to be ; the results are very striking. It will be noted that the sample originally contained bacillus coli communis in such numbers as to preponderate over all the others in the original analysis. The emulsion was placed in the sun about noon of the 2nd September and by the end of the 3rd, that is to say, presumably after about 18 hours' exposure to sunlight, all the coli communis had disappeared, and were never found in the emulsion again. These coli being killed off, an entirely new set of organisms appeared in the analysis, namely, coscoroba, neapolitanus, pneumoniæ and Nos. 100 and 101. By the

Experiment No. 1.

Date.	Experiment No. 1.	Number.	Sacch.	Dulcit.	Adonit.	Inulin.	V. and P.	Motility.	Bacillus.	Group.	Remarks. Temperature.
2nd Sept. 1908 ..	Before exposure	1	–	+	–	–	–	–	Coli Com.* ..	II	31° C.
		2	–	+	–	–	–	–	Do. ..	,,	
		3	–	+	–	–	–	–	Do. ..	,,	
		4	–	+	–	–	–	–	Do. ..	,,	
		5	–	+	–	–	–	–	Do. ..	,,	
		6	–	+	–	–	–	–	Do. ..	,,	
		7	–	+	–	–	–	–	Do. ..	,,	
		8	–	+	–	–	–	–	Do. ..	,,	
		9	–	+	–	–	–	–	Do. ..	,,	
		10	–	+	–	–	–	–	Do. ..	,,	
2nd do. ..	Sample 2. One hour's exposure + in 5 cc. of water.	..	..	..	..	..	..	..	....	..	40° C.
	,, 3. Two hours' exposure + in 5 cc.	..	..	..	..	..	..	..	....	..	37° C.
	,, 4. Three ,, ,, + ,,	..	..	..	..	..	..	..	....	..	35° C.
	,, 5. Five ,, ,, + ,,	..	..	..	..	..	..	..	....	..	31° C.
3rd do. ..	,, 6. End of day + in 5 cc. of water.	1	+	–	+	–	–	+	No. 100 ..	IV	
		2	+	+	+	–	–	+	Pneumoniæ ..	III	
		3	+	+	+	–	–	–	Do. ..	III	
		4	+	–	+	–	–	–	No. 101 ..	IV	
		5	+	+	–	–	–	–	Neapolitanus ..	III	
		6	+	–	–	–	–	+	Coscoroba ..	IV	
		7	+	–	–	–	–	–	Do. ..	IV	
		8	+	–	+	–	–	–	No. 101 ..	IV	
		9	+	–	+	–	–	–	Do. ..	IV	
		10	+	–	–	–	–	–	Coscoroba ..	IV	
4th do. ..	,, 7. End of day + in 5 cc. of water.	..	..	..	..	..	..	..	....	..	30° C.

Experiment No. 1—Contd.

Date.	Experiment No. 1.	Number.	Sacch.	Dulcit.	Adonit.	Inulin.	V. and P.	Motility.	Bacillus.	Group.	Remarks. Temperature.
5th Sept. 1908 ..	Sample 8. End of day + in 5 cc. of water.	1	+	–	+	–	+	–	Lactis Ærogenes	IV	33° C.
		2	+	–	+	–	+	–	Do.	..	
		3	+	–	–	–	+	–	Coscoroba ..	..	
		4	+	–	+	–	+	–	Lactis Ærogenes	..	
		5	+	+	+	–	+	–	No. 67 ..	III	
		6	+	–	+	–	+	–	Lactis Ærogenes	IV	
		7	+	–	+	–	–	–	Do.	..	
		8	+	–	–	–	–	–	Coscoroba ..	..	
		9	–	–	+	–	–	+	Acidi Lactici ..	I	
		10	–	–	–	–	–	–	Grunthal ..	I	
		11	+	–	–	–	+	–	Cloacæ ..	IV	
7th do. ..	,, 9. End of day + in 5 cc. of water.	1	–	+	+	–	–	–	No. 33 ..	II	32° C.
		2	–	+	+	–	–	+	Do. ..	II	
		3	–	–	–	–	–	–	Grunthal ..	I	
		4	+	–	–	–	–	–	Coscoroba ..	IV	
		5	–	+	+	–	–	–	No. 33 ..	II	
		6	+	+	+	–	–	–	Pneumoniæ ..	III	
		7	–	–	–	–	–	–	Grunthal ..	I	
		8	–	–	–	–	–	–	Do. ..	I	
		9	–	–	+	–	–	–	Acidi Lactici ..	I	
		10	–	–	+	–	–	+	Do. ..	I	
		11	+	–	+	–	–	+	No. 100 ..	IV	
		12	+	–	–	–	–	–	Coscoroba ..	IV	
8th do. ..	,, 10. End of day + in 5 cc. of water	..	..	..	..	..	..	..	....	..	30° C.
9th do. ..	,, 11. ,, ,, + ,, ,,	..	..	..	..	..	..	..	....	..	31° C.
10th do. ..	,, 12. ,, ,, + ,, ,,	..	..	..	..	..	..	..	....	..	30° C.

Experiment No. 1—Contd.

Date.	Experiment No. 1.	Number.	Sacch.	Dulcit.	Adonit.	Inulin.	V. and P.	Motility.	Bacillus.	Group.	Remarks. Temperature.
11th Sept. 1908. ..	Sample 13. End of day + in 5 cc. of water.	1	+	–	+	–	–	–	No. 101 ..	VI	30° C.
		2	+	–	+	–	–	–	Do. ..	V	
		3	+	–	+	–	–	–	Do. ..	VI	
		4	+	–	+	–	–	–	Do. ..	VI	
		5	+	–	+	–	–	–	Do. ..	VI	
		6	+	–	+	–	–	–	Do. ..	VI	
		7	+	–	–	–	–	–	Do. ..	VI	
		8	+	–	+	–	–	–	Do. ..	VI	
		9	+	–	+	–	–	–	Do. ..	VI	
		10	+	–	+	–	–	–	Do. ..	VI	
		11	+	–	+	–	+	+	Lactis Ærogenes	VI	
		12	+	–	+	–	–	–	No. 101 ..	VI	
12th do. ..	,, 14. End of day + in 5 cc. of water	..	..	..	..	..	..	..	....	..	30° C.
14th do. ..	,, 15. End of day + in 5 cc. ..	1	+	–	–	–	–	–	Coscoroba ..	VI	31° C.
		2	+	–	+	–	–	–	No. 101 ..	VI	
		3	+	–	+	–	–	–	Do. ..	VI	
		4	+	–	+	–	–	–	Do. ..	VI	
		5	+	–	+	–	–	–	Do. ..	VI	
		6	+	–	+	–	–	–	Do. ..	VI	
		7	+	–	+	–	–	–	Do. ..	VI	
		8	+	–	+	–	–	–	Do. ..	VI	
		9	+	–	+	–	–	–	Do. ..	VI	
		10	+	–	+	–	–	–	Do. ..	VI	
		11	+	–	+	–	–	–	Do. ..	VI	
		12	+	–	+	–	–	–	Do. ..	VI	
15th do. ..	,, 16. End of day + in 5 cc. of water	..	..	..	..	..	..	..	....	..	....
16th do. ..	,, 17. ,, ,, + ,, ,,	..	..	..	..	..	..	..	....	..	....
17th do. ..	,, 18. ,, ,, sterile ..	..	..	..	..	..	..	..	....	..	....
18th do. ..	,, 19. ,, ,, ,, ..	..	..	..	..	..	..	..	....	..	....

evening of the 5th a further change had come over the organisms in the emulsion, bacillus lactis ærogenes largely preponderated, acidi lactici, Grunthal and cloacæ began to appear, and another organism No. 67 was present for the first time. A sample taken on the evening of the 7th shows still further alterations. A new organism, namely, No. 33 which, it should be noticed, belongs to MacConkey's group No. 2, and possesses reactions almost identical with those of coli communis itself, appeared for the first time. Unfortunately for three days, only the daily investigation as to the presence of fæcal organisms in 5 c.c. was taken; on two more occasions the operation of putting the organisms through the sugars was carried out. But these results show that organism No. 101 preponderated and outlived most of those above-mentioned. These results, incomplete as they are, give a fair idea of the order in which the organisms died off under the action of the sunlight. The important point to notice is, that in a fæces crowded with coli communis, these organisms had entirely disappeared in less than eighteen hours, so that the various other more resistant types were easily isolated.

Experiment No. 2.

Calf Dung—7-3-1910.

Taken at 11-45 A.M., before exposure (Lactose fermenters in ·0001 cc.) ..	Vesiculosus ..	5
	Schäfferi ..	5
,, 12 noon	Vesiculosus ..	9
	L. ærogenes ..	1
,, 12-15 P.M.	Vesiculosus ..	9
	L. ærogenes ..	1
,, 12-30 ,, ...	Vesiculosus ..	6
	L. ærogenes ..	2
	Cloacæ ..	1
	Mutabilis ..	1
,, 12-45 ,, ...	Vesiculosus ..	6
	Coscoroba ..	1
	——— ..	3
,, 1 ,, ...	Vesiculosus ..	2
	L. ærogenes ..	4
	Gasiformans ..	4
,, 1-15 ,, ...	L. ærogenes ..	8
	Gasiformans ..	2

Experiment No. 2—Contd.

Taken at	1-30 P.M.	L. ærogenes	3
		Gasiformans	7
		Cloacæ	2
,,	2 ,,	L. ærogenes	10
,,	2-15 ,,	L. ærogenes	10
,,	2-30 ,,	L. ærogenes	10
,,	2-45 ,,	L. ærogenes	10
,,	3-10 ,,	Sterile.	
,,	4 ,,		
,,	5 ,,		

Note.—The presence of B. Schäfferi in the emulsion and its rapid disappearance—the appearance of bacillus cloacæ as the experiment proceeds and also that of lactis ærogenes which survives all others. This experiment is not so satisfactory as many of the others because the disappearance of all bacilli is so rapid.

Experiment No. 3.

Calf Dung—17-3-1910.

Taken at	11-30 A.M., before exposure	Vesiculosus	4
		Acidi lactici	1
		Coscoroba	2
		Lactis ærogenes	1
		Gasiformans	1
		Pneumoniæ	1
,,	11-45 after 15 minutes' exposure	Vesiculosus	4
		Acidi lactici	1
		Coscoroba	3
		Pneumoniæ	2
,,	12-20 P.M.	Vesiculosus	7
		Mutabilis	1
		Acidi lactici	1
		Lactis ærogenes	1
,,	1-40 ,,	Vesiculosus	6
		Acidi lactici	3
		Lactis ærogenes	1
,,	2-20 ,,	Vesiculosus	6
		Mutabilis	1
		Coscoroba	2
		No. 67	1
,,	2-55 ,,	Vesiculosus	4
		Lactis ærogenes	5
		Neapolitanus	1
,,	5 ,,	Vesiculosus	3
		Lactis ærogenes	4
		No. 67	3

Experiment No. 3.—Contd.

Taken at 11-30 A.M., on 18-3-1910	Vesiculosus Grunthal	7 3
,, 12 noon	Vesiculosus Coscoroba	6 4
,, 12-40, 2 P.M., 2-30 P.M., 5 P.M.	Sterile in 20 cc.	

Note.—The very rich mixture at the commencement of the experiment and the gradual weeding out of the varieties till Vesiculosus and Coscoroba remained at the end.

Experiment No. 4—*Cowdung.*

Emulsion before exposure contained	1 coli communis, 1 cloacæ, 4 coscoroba, 4 Grunthal.
After 1 hour's exposure showed	3 coli communis, 3 Grunthal, 4 coscoroba.
,, 2 hours' ,, ,,	8 Grunthal, 1 coscoroba, 1 cloacæ.
,, 3 ,, ,, ,,	6 Grunthal, 2 coscoroba, 2 No. 100.
,, 5 ,, ,, ,,	2 Grunthal, 8 coscoroba.
At 1 o'clock on the 2nd day (after about 10 hours' exposure)	10 Grunthal.
At 5 on the second day	10 do.

Subsequent samples contained no organisms in 20 cc.

Note.—Coli communis survived one hour, but not 2 hours' exposure to sun; late appearance of bacillus cloacæ; final bacillus that remained was bacillus Grunthal; even this disappeared after about 3 days' exposure.

In the following experiments a single specimen of cow-dung was taken, but a layer of sterile sand was placed at the bottom of the dish:—

Experiment No. 5.

Emulsion before exposure contained	5 lactis ærogenes, 2 No. 100, 3 No. 67.
After 1 hour's exposure showed	5 lactis ærogenes, 3 No. 67, 2 No. 100.
,, 2 hours' ,, ,,	7 lactis ærogenes, 2 No. 100, 1 Grunthal.
,, 3 ,, ,, ,,	10 lactis ærogenes.
,, 5 ,, ,, ,,	3 lactis ærogenes, 3 No. 67, 2 cloacæ, 1 Grunthal.
Noon of the second day (after 10 hours' exposure) showed	3 lactis ærogenes, 4 cloacæ, 1 neapolitanus, 2 coscoroba.
Evening of second day showed	5 lactis ærogenes, 3 cloacæ, 1 coscoroba, 1 neapolitanus.

Evening of third day and subsequent samples were sterile in 20 cc.

Note.—This experiment is interesting on account of the preponderance of lactis ærogenes from start to finish, showing that these organisms are to be looked upon as one of the fairly resistant ones. Note also the gradual increase of bacillus cloacæ as the experiment proceeds. Bacilli Nos. 100 and 67 were present at the beginning and persisted for a considerable length of time ; unfortunately the experiment was spoilt by our not taking sufficient samples on the third day to observe the last surviving organisms.

Experiment No. 6—Cowdung.

In order to provide a larger number of varieties a mixture of 10 samples of cowdung were taken and the emulsion was exposed in the ordinary way :—

Emulsion before exposure contained	1 coli communis, 2 coscoroba, 4 Grunthal, 1 cloacæ, 1 acidi lactici.
After 1 hour's exposure showed	5 Grunthal, 1 neapolitanus, 3 No. 101, No. 6.
,, 2 hours' ,, ,,	4 Grunthal, 1 lactis ærogenes, 2 cloacæ, 1 neapolitanus, 1 coscoroba, 1 No. 67.
,, 3 ,, ,, ,,	3 Grunthal, 1 coscoroba, 2 lactis ærogenes, 2 No. 67, 1 No. 101, 1 No. 6.
Noon on the second day showed	5 cloacæ, 3 lactis ærogenes, 2 No. 101.
At 3 ,, ,,	10 No. 67.
At 5 ,, ,,	10 cloacæ.

Noon of the third day and subsequent samples were sterile in 20 cc.

Note.—Coli communis did not survive after one hour's exposure ; bacillus grunthal did not in this mixture persist till the end, but appears to have disappeared sometime prior to noon of the second day ; bacillus No. 67 and bacillus cloacæ both appeared to survive till quite late ; the rare organism No. 6 was present in this mixture and survived after three hours' exposure to sun.

Experiment No. 7.

A single sample of human fæces with a layer of sand placed at the bottom of the dish :—

Emulsion before exposure contained	3 lactis ærogenes, 2 Grunthal, 1 No. 67, 4 acidi lactici.
After 1 hour's exposure showed	1 coli communis, 2 acidi lactici, 1 No. 67, 6 Grunthal.
,, 2 hours' ,, ,,	1 lactis ærogenes, 1 acidi lactici, 1 cloacæ, 1 coscoroba, 6 Grunthal.

Experiment No. 7—Contd.

Noon of the second day (after 8 hours' exposure) showed	1 lactis ærogenes, 1 coli communis, 2 acidi lactici, 6 Grunthal.
Evening of the second day showed	1 acidi lactici, 1 neapolitanus, 3 coscoroba, 5 Grunthal.
Noon of the third day showed	7 coscoroba, 1 cloacæ, 2 Grunthal.
Evening of the third day showed	3 acidi lactici, 5 coscoroba, 2 Grunthal.
,, fourth ,,	3 acidi lactici, 3 coscoroba, 3 Grunthal, 1 cloacæ.
,, fifth ,,	3 Grunthal, 5 coscoroba, 1 acidi lactici, 1 pneumoniæ.
,, sixth ,,	6 Grunthal, 1 cloacæ, 3 coscoroba.
,, seventh ,,	7 Grunthal, 2 coscoroba, 1 neapolitanus.

Subsequent samples proved sterile in 20 cc.

Note.—Disappearance of organisms less rapid than usual due to the presence of sand; much later disappearance of coli communis, namely, at 8 hours; no subsequent appearance after this. This delayed disappearance is due to the presence of sand in the bottom of the dish; the usual appearance and disappearance of cloacæ, lactis ærogenes, and particularly coscoroba, which at one time decidedly predominated; increase in number of Grunthal for the first 4 or 5 analyses, then slight decrease followed by final survival of these organisms; coscoroba and neapolitanus in small numbers also appeared towards the end.

Experiment No. 8.

Mixture of 5 samples of human fæces with a layer of sand at the bottom:—

Emulsion before exposure contained	1 coli communis, 3 acidi lactici, 1 cloacæ, 5 Grunthal.
After 1½ hours' exposure showed	1 coli communis, 5 Grunthal, 3 acidi lactici, 1 cloacæ.
,, 2½ ,, ,, ,,	1 acidi lactici, 2 No. 67, 7 Grunthal,
,, 4½ ,, ,, ,,	2 acidi lactici, 1 cloacæ, 7 Grunthal.
Noon of the third day	2 coli communis, 3 acidi lactici, 1 coscoroba, 4 Grunthal.
Evening of the third day showed	9 Grunthal, 1 cloacæ.
,, fourth ,,	8 Grunthal, 2 cloacæ.
,, fifth ,,	5 acidi lactici, 5 Grunthal.
,, sixth ,,	3 acidi lactici, 7 Grunthal.
,, seventh ,,	Sterile.

Note.—Very slight variation in number of Grunthal from beginning to end of the experiment, the persistence of coli till the end of the second day, but not longer (as in other experiments); comparatively late persistence of acidi lactici; usual appearance and disappearance of No. 67 and cloacæ. Note the alteration in numerical relation of bacilli to one another in the first 1½ hours is *nil.*

Experiment No. 9.

A single sample of human fæces with a layer of sterile sand at the bottom of the dish:—

Emulsion before exposure contained ..	3 acidi lactici, 2 coscoroba, 4 Grunthal, 1 No. 6.
After 1 hour's exposure showed ..	1 lactis ærogenes, 2 coscoroba, 1 No. 75, 6 Grunthal.
,, 2 hours' ,, ,, ..	3 lactis ærogenes, 8 Grunthal, 2 No. 75.
,, 3 ,, ,, ,, ..	2 lactis ærogenes, 2 cloacæ, 1 No. 75, 2 coscoroba, 3 Grunthal.
,, 5 ,, ,, ,, ..	7 Grunthal, 1 coscoroba, 1 No. 67, 1 No. 6.
Noon of the second day (after 11 hours' exposure)	7 Grunthal, 2 coscoroba, 1 lactis ærogenes.
Evening of the second day ..	6 Grunthal, 3 acidi lactici, 1 No. 101.
Noon of the third day	5 acidi lactici, 5 Grunthal.
Evening of the fourth day ..	4 acidi lactici, 1 cloacæ, 5 Grunthal.
,, fifth ,, ...	3 acidi lactici, 7 Grunthal.
,, sixth ,, ...	8 Grunthal, 2 No. 101.
,, seventh ,, ...	No organisms.

Note.—Persistence of acidi lactici from start till quite late; appearance and disappearance of lactici ærogenes; appearance and disappearance of organism No. 75; presence of rare organism No. 6 and its persistence for five hours at least; final survival of bacillus Grunthal.

Besides exposing to the sunlight emulsions of fæces a few experiments were done on some special samples of water only.

Experiment No. 10.

The river Adyar flows close to the King Institute. It is very shallow. It is considerably polluted all along the banks and very highly polluted just below the bridge where many hundreds of washermen wash the clothes of the inhabitants of the city:—

Experiment No. 10—Contd.

The water before exposure contained ..	1 lactis ærogenes, 2 coscoroba, 1 neapolitanus, 1 cloacæ, 2 acidi lactici, 3 Grunthal.
After 1 hour's exposure showed ..	2 lactis ærogenes, 2 cloacæ, 1 neapolitanus, 1 coscoroba, 3 Grunthal, 1 No. 101.
,, 2 hours' ,, ,, ..	3 lactis ærogenes, 6 coscoroba, 1 Grunthal.
,, 3 ,, ,, ,, ..	1 lactis ærogenes, 3 No. 67, 6 Grunthal.
,, 4 ,, ,, ,, ..	2 lactis ærogenes, 3 cloacæ, 5 No. 67.
,, 5 ,, ,, ,, ..	10 No. 101.
,, 6 ,, ,, ,, ..	9 cloacæ, 1 No. 67.

Noon of the second day (after 12 hours' exposure) sterile, no lactose fermenters.

Note.—The original water is very rich in different species; persistence of lactis ærogenes till near the end; appearance of organism No. 67 and particularly No. 101 late on in the exposure; final survival of cloacæ.

Now when an emulsion very rich in fæcal organisms is exposed to the sun, within the first hour or two (according to the clearness of the liquor, and the power of the sun), a very large number of these bacilli die off, and the more resistant organisms begin to appear in the test portions. It must be carefully understood that if the organisms A., B., C. (A. being a delicate organism, B. one of medium resisting power and C. a resistant one) be exposed to the sun, it is not maintained that all the A's must disappear before any of the B's begin to die, or that the whole lot of organism B. must have been killed before any of C. die off. There must be a great deal of overlapping, especially among organisms whose power of resistance is nearly equal. Again if 100 pure cultures of A. B. and C. were all exposed to the sun under similar circumstances, it is not maintained that all the A's would die out before any of the B's or C's. In a hundred such cultures, from different strains, a proportion of the B's might die off before all the A's were extinct; yet the conclusion to be drawn from the whole experiment would not be altered very materially, *viz.*, that the majority of A. was more susceptible to the influence of the sun than the majority of B. and C. If, however, two very extreme cases were taken, such as a very delicate organism and a very resistant one, it is highly probable that all the delicate organisms would have disappeared before any great number of the very resistant organisms had succumbed to the action of the sun.

It may be objected that, if we intend to formulate any law as to the viability of bacilli, this law must obviously apply to all specimens of the same variety in whatever situation they may be found in nature, otherwise the statement is of no value in itself. We have no intention of laying down any hard and fast rules, for our experience teaches us that such do not exist in nature. We are quite content with the very widest generalization, provided these are based on observations from nature, and on the results of experiments carried out as nearly as possible under natural conditions. Imperfect as these experiments are, they are amply sufficient to prove the correctness of certain wide conclusions. These conclusions may be of great importance to the water analyst.

Experiment No. 11.

A mixture of five samples of human fæces—

Emulsion before exposure contained	1 coscoroba, 9 Grunthal.
After 1 hour's exposure showed	4 coli communis, 2 Grunthal, 1 lactis ærogenes, 2 neapolitanus, 1 No. 101.
,, 2¼ hours' ,, ,,	4 cloacæ, 3 coscoroba, 1 lactis ærogenes, 1 acidi lactici.
,, 3¼ ,, ,, ,,	8 cloacæ, 2 coscoroba.
,, 4½ ,, ,, ,,	7 cloacæ, 2 coscoroba, 1 Grunthal.
Noon of second day showed (after 10 hours' exposure)	1 cloacæ, 1 coscoroba, 1 No. 6, 7 Grunthal.
At 5 on the second day showed	10 Grunthal.

Subsequent samples proved sterile in 20 cc.

Note.—The prevalence of Grunthal to commence with; appearance of coli communis after 1 hour's exposure and disappearance entirely before 2 hours; usual appearance of cloacæ and great predominance until near the end when bacillus Grunthal survived all others; important comments are made on this result in the succeeding part of this chapter.

There is another point that requires discussion. In the results given in Experiment 11, on the emulsion being put into the sun 9 Grunthal and 1 coscoroba were present.

One hour afterwards the analysis reveals an entirely different state of affairs. Only 2 Grunthal persist, 4 coli communis and sundry other organisms have made their appearance. If a large number of Grunthal had not disappeared during the first hour, they would have still predominated in the second, third and fourth analyses to the exclusion of other varieties. Consequently it is certain that quite a fair percentage of this bacillus Grunthal disappeared within the first hour. But as the experiment proceeds the usual state of affairs declares itself, namely, cloacæ begins slowly to predominate, and continues to do so up to within the last two analyses, when bacillus Grunthal again comes to the front, and in the final analysis not a single other organism could be isolated. It should be observed that this emulsion was made from 5 different specimens of human fæces, and a layer of sand was placed at the bottom of the dish. Thus we are absolutely forced to the conclusion, that, although a large number of the organisms, included in the term bacillus Grunthal, were sufficiently susceptible to die off in one hour's exposure, the remainder were very resistant. Now it may be argued, that considering bacillus Grunthal was by far the most numerous organism present at the beginning of the experiment, the following explanation would completely satisfy all the facts observed. Let it be granted that in a large number of individual bacilli of the same species resistant and delicate specimens exist, then any organism that preponderates at the beginning of the experiment will survive to the end, because the more organisms there are at the commencement, the greater is the chance of resistant ones being included amongst the number of those experimented on. It must be admitted that such explanation would account for all the results in Experiment 11. But if this rule be applied universally, the results obtained would not be as uniform as they are. Further, other experiments disprove this theory. Thus, in the case of bacillus Grunthal; other experiments could be given showing the steady increase in the number from beginning to end. In Experiment No. 12, note not a single Grunthal was isolated in the first analysis; yet it survived all others.

Experiment No. 12—*Cowdung.*

Emulsion before exposure contained ..	4 coscoroba, 1 cloacæ, 1 coli communis, 3 neapolitanus, 1 acidi lactici.
After 1 hour's exposure showed ..	7 coscoroba, 1 Grunthal, 1 cloacæ, 1 neapolitanus.
,, 4 hours' ,, ,, ..	7 lactis ærogenes, 3 Grunthal.
Noon of second day	10 Grunthal.
Evening of second day	10 do.
Noon of third day	10 do.
Evening of third day	10 do.
Noon of fourth day	10 do.
Evening of fourth day	10 do.

In subsequent 20 cc. samples there were no organisms.

Note.—Disappearance of coli communis after 1 hour's exposure ; fairly rapid disappearance of neapolitanus ; these organisms did not survive longer than a few hours ; appearance of bacillus Grunthal after one hour though it was not detected in the sample before exposure ; appearance of lactis ærogenes sometime before 4 hours' exposure, but its disappearance before noon of second day ; the longer persistence of bacillus Grunthal as compared with other organisms.

Experiment No. 13—*Cowdung.*

Emulsion before exposure contained ..	3 coscoroba, 2 coli communis, 2 acidi lactici, 1 Grunthal.
After 1 hour's exposure showed ..	1 coli communis, 7 coscoroba, 2 Grunthal.
,, 2 hours' ,, ,, ..	3 coscoroba, 6 Grunthal, 1 cloacæ.
,, 4 ,, ,, ,, ..	10 Grunthal.

The emulsion became free from lactose fermenters by noon of the second day.

Note.—That bacillis coli communis survived for one hour, but not for two ; that bacillus Grunthal was the last survivor ; the late appearance of cloacæ.

If further proof be necessary, the following points are quite convincing : (1) In Experiment No. 1, the preponderating organism was coli communis ; it disappeared entirely before the second analysis was taken, and never reappeared ; (2) in very few of the emulsions before exposure is bacillus cloacæ shown to be present. In nearly every sample bacillus cloacæ appeared later on, persisted for a certain length of time, and either remained to the end or disappeared just before bacillus Grunthal. These two important facts are, we consider, sufficient to dispose of a general assumption that the predominating bacillus in an emulsion at the commencement of an experiment will, other things being equal, be the last to survive.

Before finally leaving this subject we wish to emphasise, what has already been laid down, *viz.*, that we do not state that there is a perfectly fixed degree of power of resistance to sunlight in all specimens of any one species, but simply, that a certain proportion of one organism resist sunlight longer than a certain proportion of another, both being placed under similar conditions.

Making due allowance for the above mentioned objections we consider that a few outstanding facts are apparent to any fair-minded individual. Of these perhaps the most important is the consistent rapid disappearance of coli communis. In support of this Experiments 1 and 2 are very convincing. In the first of these we start out with an enormous preponderance of these bacilli, and within a very short time they have disappeared, and it has become possible to isolate other varieties. Another equally interesting point is the tendency for the last organism to live being either one Gruntthal group or B. cloacæ. Practically all our experiments show that it is quite common for these varieties to survive all others. We are very anxious not to overestimate the value of these results, or to draw more conclusions from them than are legitimate, but the following broad conclusions appear to be justifiable :—

(1) That the sun has a very powerful action in destroying all fæcal organisms in water, particularly when they are "naked" and not surrounded by mucus derived from the intestine ;

(2) That all fæcal organisms do not possess the power of resisting the action of sunlight to an equal degree ;

(3) That it is possible to divide fæcal organisms, with a reasonable degree of accuracy, into the following classes :—

(*i*) The delicate organisms or those that are very susceptible to the action of the sunlight (*vide* the remarks on coli communis) ;

(*ii*) An intermediate class, containing a very large number of organisms which occupy a position midway between the two extremes ; and

(*iii*) The resistant organisms, or those capable of resisting the action of sunlight for considerable length of time (*vide* the remarks on cloacæ, Grunthal, etc.)

It is not proposed to discuss the position of any particular species of organisms in these three classes, until the conclusions arrived at in the further work on this subject have been considered.

CHAPTER VIII.

THE EFFECT OF SUNLIGHT CONTINUED : THE RESULTS OBTAINED FROM THE RED HILLS LAKE NEAR MADRAS.

IT may possibly be objected that the experiments described in the last chapter, designed to show the action of light on fæcal organisms, are of a somewhat artificial nature, and therefore conclusions deduced from the results cannot apply to strictly natural conditions. In order to meet this objection, it was decided that the effects of sunlight and storage should be studied in a large lake. The Red Hills Lake was selected because it was within an easy distance of the laboratory, and because it is the source of supply for the town of Madras. The lake is a very large sheet of water, being about 14 miles in circumference; it is supplied by 4 or 5 channels, most of which discharged into the lake for about 8 to 10 months of the year; it is primarily an irrigation tank and receives considerable quantities of ''paddy land'' water, so that it cannot be said to be strictly preserved from contamination.

Table No. VIII (*a*) gives the results of the weekly samples. These we will now discuss. Sample No. 1 was taken at the burst of the monsoon, and shows a large number of oxytocus perniciosus, a bacillus which is always associated with heavy monsoon weather.

Samples Nos. 1, 2, 3 and 4 show that practically the whole volume of water in the lake is full of contamination washed off the land, and that up to this point, the only sign that any purification has commenced is the rapid disappearance of oxytocus perniciosus and organism No. 69. During the 3 weeks from October 28th to November 23rd a very large quantity of rain fell, about 30 inches, and for a considerable portion of this period the sky was very overcast. Further, up till nearly the end of November the supply channels were bringing in a very large volume of highly polluted water washed off ''paddy fields;'' analysis 4 (*a*) shows that even the surface sample contained a considerable amount of silt.

Tabular Statement of Results of Analyses of Red Hills Lake, 1908. TABLE VIII (*a*).

Sample number.	Date of taking sample.	Sample taken from	Total colonies Agar 37° C.	Fœcal bacilli in	Organisms present and number.	Depth of water in lake.	Rainfall and other remarks.
I	Oct. 25	Surface 1′ deep.	..	·1 c.c.	5 Oxytocus perniciosus, 3 No. 69, 2 No. 105.	9·1′	Lake rose on account of rain about 4½ feet in one night.
2	Oct. 31	Surface.	..	1 c.c.	9 Grunthal, 1 cloacæ ..	11·9′	Due to more rain.
2*b*	,,	Bottom 14′.	..	1 c.c.	2 Acidi lactici, coli communis, 1 Grunthal, 2 cloacæ, 1 No. 101, 1 No. 69.	..	
3*a*	Nov. 11	Surface.	..	1 c.c.	3 Grunthal, 1 coscoroba, 2 cloacæ, 1 lactis ærogenes, 3 No. 101.	12·9′	Due to continued rainfall up to November 7th.
3*b*	,,	Bottom 14′.	..	1 c.c.	2 Grunthal, 3 lactis ærogenes, 1 cloacæ, 2 No. 101, 1 No. 67, 1 No. 75.		
4*a*	Nov. 23	Surface.	150	1 c.c.	10 Cloacæ	12·25′	Total sediment in surface ·55 part per 100,000.
4*b*	,,	Bottom 11′.	440	1 c.c.	9 Lactis ærogenes, 1 pneumoniæ.	..	No rain.
5*a*	Nov. 29	Surface.	160	5 c.c.	9 Lactis ærogenes, 1 coscoroba.	12·4′	No rain.
5*b*	,,	Bottom 6′.	510	1 c.c.	9 Cloacæ, 1 No. 75 ..	..	
5*c*	,,	Middle 3′.	190	1 c.c.	7 Lactis ærogenes, 1 cloacæ, 2 No. 101.	..	
5*a*	Dec. 7	Surface.	290	5 c.c.	5 Coli communis, 4 cloacæ, 1 neapolitanus.	12·3′	Heavy rain fell about 5th December.
6*b*	,,	Bottom 6½′.	360	5 c.c.	1 Coli communis, 6 Grunthal, 2 cloacæ, 1 No. 37.	..	Analyses of channels supplying the lake taken at this time shows that some of these channels are discharging in very polluted water. Sediment of surface ·4 part per 100,000.
6*c*	,,	Middle 3′.	430	1 c.c.	2 Coli communis, 1 Grunthal, 5 coscoroba, 2 lactis ærogenes.	..	

TABLE VIII (*a*)—*contd.*

Sample number.	Date of taking sample.	Sample taken from	Total colonies Agar 37° C.	Fœcal bacilli in	Organisms present and number.	Depth of water in lake.	Rainfall and other remarks.
7*a*	Dec. 13	Surface.	1,110	50 c.c.	*Nil*	..	
7*b*	,,	Bottom 11′.	1,200	10 c.c.	9 Cloacæ, 1 coscoroba	..	
7*c*	,,	Middle 5′.	1,420	20 c.c.	6 Lactis ærogenes, 4 Grunthal.	..	No rain.
8*a*	Dec. 18	Surface.	360	50 c.c.	*Nil*	12·23′	No rain.
8*b*	,,	Bottom 8′.	580	10 c.c.	10 Cloacæ	..	Sediment in surface sample ·35 parts per 100,000.
8*c*	,,	Middle 4′.	850	1 c.c.	9 Lactis ærogenes, 1 No. 101.	..	
9*a*	Jan. 6	Surface.	620	1 c.c.	8 Acidi lactici, 2 Grunthal	..	Heavy rain fell first week of the year; a polluted layer of water on the surface.
9*b*	,,	Bottom 18′.	360	10 c.c.	5 Cloacæ, 5 No. 6	..	
9*c*	,,	Middle 8′.	340	5 c.c.	9 Grunthal, 1 acidi lactici	..	
10*a*	Jan. 14	Surface.	1,260	1 c.c.	5 Acidi lactici, 2 coscoroba, 3 No. 101.	12·75′	No rain, but rise due to last fall.
10*b*	,,	Bottom 10′.	1,890	1 c.c.	9 Coscoroba, 1 neapolitanus	..	
10*c*	,,	Middle 5.	3,150	1 c.c.	5 Grunthal, 3 coscoroba, 1 cloacæ, 1 neapolitanus.	..	
11*a*	Jan. 20	Surface.	690	20 c.c.	8 Coscoroba, 2 lactis ærogenes.	13′	No rain.
11*b*	,,	Bottom 18′.	110	50 c.c.	8 Coscoroba, 2 Grunthal	..	
11*c*	,,	Middle 9′.	630	50 c.c.	*Nil*	..	
12*a*	Jan. 26	Surface.	780	5 c.c.	8 No. 101, 2 No. 69	..	
12*b*	,,	Bottom 14′.	860	5 c.c.	8 Cloacæ, 2 lactis ærogenes	13′	No rain; irrigation water probably admitted.
12*c*	,,	Middle 7′.	..	5 c.c.	10 Grunthal	..	
13*a*	Feb. 3	Surface.	68	50 c.c.	*Nil*	12·7′	No rain.

13*b*	Feb. 3	Bottom 16′.	150	5 c.c.	8 Cloacæ, 1 lactis ærogenes, 1 No. 73.	..	
13*c*	,,	Middle 8′.	56	50 c.c.	6 Acidi lactici, 1 Grunthal, 1 No. 6, 2 No. 101.	..	
14*a*	Feb. 9	Surface.	350	10 c.c.	5 Cloacæ, 5 No. 67 ..	12·54′	No rain.
14*b*	,,	Bottom 18′.	420	5 c.c.	4 Cloacæ, 6 No. 73 ..	..	
14*c*	,,	Middle 8′.	380	10 c.c.	10 Grunthal..	..	
15*a*	Feb. 15	Surface.	360	20 c.c.	7 Lactis ærogenes, 2 No. 67, 1 cloacæ.	12·4′	
15*b*	,,	Bottom 16′.	480	1 c.c.	1 Grunthal, 7 cloacæ, 2 coscoroba.	..	
15*c*	,,	Middle 8′.	280	20 c.c.	Plate spoilt	..	
16*a*	Mar. 1	Surface.	320	50 c.c.	*Nil*	12′	
16*b*	,,	Bottom 14′.	380	·1 c.c.	3 Cloacæ, 6 lactis ærogenes, 1 No. 101.	..	
16*c*	,,	Middle 7′.	510	·1 c.c.	4 Coscoroba, 1 pneumoniæ, 2 No. 6, 1 No. 67, 1 No. 101, No. 73.	..	
17*a*	Mar. 8	Surface.	310	50 c.c.	*Nil.*	11·5′	
17*b*	,,	Bottom 15′.	410	·1 c.c.	6 Lactis ærogenes, 1 coscoroba, 3 No. 101.	..	
17*c*	,,	Middle 7′.	3,501	·1 c.c.	9 Cloacæ, 1 coscoroba ..	..	
18*a*	Mar. 17	Surface.	Useless plate.	50 c.c.	10 Lactis ærogenes ..	..	
18*b*	,,	Bottom 16′.	80	5 c.c.	8 Coscoroba, 1 cloacæ, 1 neapolitanus.	..	
18*c*	,,	Middle 8′.	120	5 c.c.	10 Coscoroba		

It should be observed that coli communis was only once isolated in this period, in spite of the fact that a constant stream of this bacterium was coming in. Note the great number of varieties present, and the truly fæcal nature of the mixture of organisms shown in each analysis.

Sample No. 5 taken on November 29th shows that the action of sunlight has already commenced ; for the surface layers of the lake are considerably purer than the bottom and middle. This improvement goes on steadily till sample No. 8 (*a*) taken on December 18th, which shows that the surface water is extremely pure. Note that with the improvement lactis ærogenes becomes common.

On December 5th a heavy downpour brought in to the lake a further rush of very highly polluted water, though it does not appear to have raised the total number of lactose fermenting organisms very much. As this new pollution was chiefly shown by the presence of coli communis, improvement in quality of the water occurred very rapidly. After the rain on December 5th, note the disappearance of lactis ærogenes, and its preponderance again a few weeks later.

The sample taken in January 6th is very unusual. In this case the surface water is distinctly inferior in quality to that in the lower depths. The reason for this is, that when the sample was taken heavy rain was falling, probably a layer of warm highly polluted surface water was lying on the surface of the lake, and thorough mixing between the purer water and this contaminated layer had not yet taken place. The sample taken a week later shows that mixing of the whole volume of water had occurred, for the lake has risen nearly six inches, and surface, middle, and lower layers are equally contaminated.

Samples 11 *a*, *b*, *c*, taken on the 28th January, are quite inexplicable and probably there is an error somewhere.

As has already been pointed out, the samples taken week by week do not show a progressive purification. There is a considerable variation, particularly between samples 15 *a*, *b*, *c*, and 16 and 17 *a*, *b*, *c*. The explanation of this irregularity is due to an in-rush of irrigation water from paddy fields down one or other of the inlet channels. In spite of this, the surface water remains always very pure.

In the table showing these analyses there is one point, which will certainly come as a surprise to anybody perusing it carefully, namely, that in a fair proportion of the samples taken, the number of water organisms and fæcal bacilli is greater in the middle layers than it is either on the surface or at the bottom. The number of instances in which this occurs is too great to be explained by the supposition that some error occurred either in taking or analysing the sample. These were taken, in every instance but one, by the same man, and from as nearly as possible the same spot in the lake, a considerable distance (600—700 yards) from the edge, all usual precautions being observed. Further examination of this point is necessary before it can be accepted as constant, or before an explanation is sought for.

One point that is amply demonstrated in these results, is the fact that there is an enormous preponderance of bacillus cloacæ in all the samples taken from the bottom of the lake. A glance at the table shows this is beyond doubt. Hence it would appear, that this organism is more resistant than others to the influences which are at work in this situation.

The rarity of bacillus coli communis on the whole is also remarkable. Only on two occasions was it found, and in only one was it present in any considerable quantity (*vide* samples 6 *a*, *b*, *c*, taken on December 7th). They were probably brought into the lake by a downpour of rain, which occurred in the very early part of December. Samples taken from some of the inflow channels, about this time, show that bacillus coli communis was present in them. It should be noted that this organism was present in all the samples, surface, middle and bottom, consequently they must have been very numerous. By December 13th (that is to say, one week afterwards) no coli communis could be found in the lake at all. The surface samples showed no fæcal organisms in 50 c.c., the bottom samples as usual showed a great preponderance of cloacæ, and the middle the usual lactis ærogenes, with some bacillus Grunthal.

The other instance when coli communis was found, was in the sample taken from the bottom on October 31st; none were found when the next sample was taken on November 11th.

As regards the other organisms commonly found in the water it will be observed that these are mostly lactis ærogenes, coscoroba, Grunthal, acidi lactici and the usual fæcal bacilli, which may be described, as of medium resistibility to the action of sunlight. It cannot be claimed, as in some of the other work, that bacillus Grunthal shows any tendency to survive other organisms.

The position of B. lactis ærogenes in these analyses is of interest. It will be noticed that this micro-organism is only found in small numbers when there is decided evidence of recent pollution, as shown by the great mixture of organisms and the presence of coli communis. But when purification has fairly started, the numbers increase, particularly in the middle layers. This would indicate that the bacillus is possessed of fair powers of resistance to sunlight, and in all probability multiplies during a certain stage of the purifying process ; and conversely, when lactis ærogenes is plentiful, a certain amount of purification has been accomplished.

The following conclusions appear to be justifiable :—

(1) That the action of the sun is powerful in destroying the fæcal organisms, and that the surface layers of any large volume of water are in consequence purer than the deeper ones. A practical point, following on this statement, is that the outlet from a reservoir should be arranged as near the surface as is possible.

(2) That these analyses show that, in the Red Hills Lake, bacillus cloacæ is by far the commonest fæcal organism isolated from the bottom.

(3) That coli communis, even in large numbers, in a huge volume of water, disappear with great rapidity. In the results obtained from the Red Hills Lake the organisms never survived longer than 6 days.

Two years after the work above described, a short series of analyses were conducted on the same lake in order further to investigate the clearing up process. These results have already been referred to in Chapter VI, in connection with the acid and gas line in glucose and lactose media. Separation of the various lactose fermenters was carried out and a few interesting points have been demonstrated. Unfortunately during the time at our disposal (which was a period of about 3 weeks, during a holiday), the lake never really became satisfactorily polluted. There was only one heavy shower, and this, although it was sufficient to add a considerable quantity of pollution, was not enough to wash the contamination from a very large area into the lake. Consequently judging the water from the point of view of lactose fermenters, the contamination was probably very little else than cowdung from the immediate surroundings of the lake. Table No. VIII(*b*) gives the analysis of water before and

TABLE VIII (*b*)

RED HILLS, SURFACE.—20-9-11.

			Glucose.		Lactose.	
1	..	20	1	*Nil*	1	*Nil*
2	..	10	2	*Nil*	2	*Nil*
3	..	5	3	*Nil*	3	*Nil*
3	..	1	3	*Nil*	1	2
3	..	·1	3	*Nil*	*Nil*	3
3	..	·05	3	*Nil*	1	2
3	..	·03	*Nil*	3	*Nil*	3
3	..	·01	*Nil*	3	*Nil*	3
3	..	·005	*Nil*	3	*Nil*	3
3	..	·003	*Nil*	3	*Nil*	3
3	..	·001	*Nil*	3	*Nil*	3
				·05		5

	Sacchar.	Dulcite.	Adonite.	Inulin.	V. & P.	Indol.	Motility.	Name or Number.
1	–	–	–	–	–	+	–	Vesiculosus.
2	+	–	–	–	–	–	–	III.
3	–	–	–	–	–	+	–	Vesiculosus.
4	–	–	–	–	–	+	–	Do.
5	+	–	–	–	–	+	–	Coscoroba.
6	–	–	–	–	–	+	–	Vesiculosus.
7	–	–	–	–	–	+	–	Do.
8	–	–	–	–	–	+	–	Do.
9	+	–	–	–	+	–	–	Cloacæ.
10	–	–	–	–	–	–	–	Vesiculosus.

Prior to pollution.

Note.—High glucose line due to great numbers of Bacillus P. and few lactose fermenters most of which are vesiculosus.

RED HILLS, SURFACE.—21-9-11.

			Glucose.		Lactose.	
1	..	20	1	*Nil*	1	*Nil*
2	..	10	2	*Nil*	2	*Nil*
3	..	5	3	*Nil*	3	*Nil*
3	..	1	3	*Nil*	3	*Nil*
3	..	.1	3	*Nil*	3	*Nil*
3	..	·05	3	*Nil*	3	*Nil*
3	..	·03	3	*Nil*	2	1
3	..	·01	3	*Nil*	1	2
3	..	·005	*Nil*	3	*Nil*	3
3	..	·003	*Nil*	3	*Nil*	3
3	..	·001	*Nil*	3	*Nil*	3
				·01		·03

	Sacchar.	Dulcite.	Adonite.	Inulin.	V. & P.	Indol.	Motility.	Name or Number.
1	–	–	–	–	–	–	–	Mutabilis.
2	+	–	–	–	–	–	–	III.
3	–	–	–	–	–	+	–	Vesiculosus.
4	+	+	–	–	–	–	–	?
5	+	–	–	–	–	–	–	III.
6	–	–	–	–	–	+	–	Vesiculosus.
7	–	–	–	–	–	+	–	Do.
8	–	–	–	–	–	+	–	Do.
9	–	–	–	–	–	+	–	Do.
10	+	–	–	–	–	+	–	Coscoroba.

Rain actually falling.

Note.—Increase in numbers of both classes and nearness of acid and gas lime in both broths.

SURFACE.—22-9-11. RAIN.

			Glucose.		Lactose.			Sacchar.	Dulcite.	Adonite.	Inulin.	V. & P.	Indol.	Motility.	Name or Number.
1	..	20	1	*Nil*	1	*Nil*									
2	..	10	2	*Nil*	2	*Nil*									
3	..	5	3	*Nil*	3	*Nil*									
3	..	1	3	*Nil*	3	*Nil*	1	+	–	+	–	+	–	–	Lactis ærogenes.
3	..	·1	3	*Nil*	3	*Nil*	2	+	–	–	–	–	–	–	III
3	..	·05	3	*Nil*	3	*Nil*	3	+	–	+	–	+	–	–	Lactis ærogenes.
3	..	·03	3	*Nil*	3	*Nil*	4	+	–	+	–	+	–	–	Do.
3	..	.01	3	*Nil*	3	*Nil*	5	+	–	+	–	+	–	–	Do.
3	..	·005	3	*Nil*	3	*Nil*	6	+	–	+	–	+	–	–	Do.
3	..	·003	3	*Nil*	3	*Nil*	7	+	–	+	–	–	–	–	Gasiformans.
3	..	·001	2	1	1	2	8	+	+	–	–	+	–	–	75.
				·001		·003	9	+	–	+	–	–	–	–	Gasiformans.
Pollution complete.							10	+	–	–	–	–	–	–	111.

immediately after pollution by the rain. It will be observed that this pollution did not add any coli communis or susceptible organisms. Consequently, it is impossible to discuss the rapidity of disappearance of this particular organism. Unfortunately, also on account of the short time available, the final analysis did not demonstrate any great prevalence of either bacillus cloacæ, vesiculosus or the Grunthal group. But the point that is strikingly demonstrated by this series is that during 10 days after pollution there is a most extraordinary prevalence of bacillus lactis ærogenes. This point will not be discussed at great length here, but it bears out the result given in the previous portion of this chapter, namely, that after pollution lactis ærogenes usually becomes prevalent for a certain length of time. A few typical analyses from these results are given in Table VIII (*c*); these demonstrate this point; it should be remembered that this organism was not present prior to the shower of rain.

TABLE VIII (*c*)

SURFACE.—26-9-11.

			Glucose.		Lactose.			Sacchar.	Dulcite.	Adonite.	Inulin.	V. & P.	Indol.	Motility.	Name or Number.
1	..	20	1	*Nil*	*Nil*	1									
2	..	10	2	*Nil*	1	1									
3	..	5	3	*Nil*	*Nil*	3	1								
3	..	1	*Nil*	3	*Nil*	3	2								
3	..	·1	*Nil*	3	*Nil*	3	3								
3	..	·05	*Nil*	3	*Nil*	3	4								
3	..	·03	*Nil*	3	*Nil*	3	5								
3	..	·01	*Nil*	3	*Nil*	3	6			could not be isolated.					
3	..	·005	*Nil*	3	*Nil*	3	7								
3	..	·003	*Nil*	3	*Nil*	3	8								
3	..	·001	*Nil*	3	*Nil*	3	9								
				5		60	10								

Note.—Great purity of water 5 days after pollution.

SURFACE.—27-9-11.

			Glucose.		Lactose.	
1	..	20	1	*Nil*	1	*Nil*
2	..	10	2	*Nil*	2	*Nil*
3	..	5	3	*Nil*	3	*Nil*
3	..	1	3	*Nil*	*Nil*	3
3	..	·1	1	2	*Nil*	3
3	..	·05	1	2	*Nil*	3
3	..	·03	*Nil*	3	*Nil*	3
3	..	·01	*Nil*	3	*Nil*	3
3	..	·005	*Nil*	3	*Nil*	3
3	..	·003	*Nil*	3	*Nil*	3
3	..	·001	*Nil*	3	*Nil*	3
			1		5	

	Sacchar.	Dulcite.	Adonite.	Inulin.	V. & P.	Indol.	Motility.	Name or Number.
1	+	−	+	−	+	−	−	Lactis ærogenes.
2	+	−	+	−	+	−	−	Do.
3	+	−	+	−	+	−	−	Do.
4	+	−	+	−	+	−	−	Do.
5	+	−	+	−	+	−	−	Do.
6	+	−	+	−	+	−	−	Do.
7	+	−	+	−	+	−	−	Do.
8	+	−	+	−	+	−	−	Do.
9	+	−	+	−	+	−	−	Do.
10	+	−	+	−	+	−	−	Do.

Note.—Increase in both classes glucose fermenters due to Bacillus P., lactose fermenters to lactis ærogenes.

SURFACE.—28-9-11.

			Glucose.		Lactose.	
1	..	20	1	*Nil*	1	*Nil*
2	..	10	2	*Nil*	2	*Nil*
3	..	5	3	*Nil*	3	*Nil*
3	..	1	3	*Nil*	3	*Nil*
3	..	·1	3	*Nil*	2	1
3	..	·05	1	2	*Nil*	3
3	..	·03	*Nil*	3	*Nil*	3
3	..	·01	*Nil*	3	*Nil*	3
3	..	·05	*Nil*	3	*Nil*	3
3	..	·003	*Nil*	3	*Nil*	3
3	..	·001	*Nil*	3	*Nil*	3
			·1		·1	

	Sacchar.	Dulcite.	Adonite.	Inulin.	V. & P.	Indol.	Motility.	Name or Number.
1	+	−	+	−	+	−	−	Lactis ærogenes.
2	+	+	+	−	−	−	−	Pneumoniæ.
3	+	+	+	−	−	−	−	Do.
4	+	+	+	−	+	−	−	67.
5	+	+	+	−	−	−	−	Pneumoniæ.
6	+	−	+	−	+	−	−	Lactis ærogenes.
7	+	−	+	−	+	−	−	Do.
8	+	−	+	−	+	−	−	Do.
9	+	−	+	−	+	−	−	Do.
10	+	−	+	−	+	−	−	Do.

Note.—Increase amongst lactose fermenters is due to multiplication of lactis ærogenes.

SURFACE.—29-9-11.

			Glucose.		Lactose.	
1	..	20	1	*Nil*	1	*Nil*
2	..	10	2	*Nil*	2	*Nil*
3	..	5	3	*Nil*	2	1
3	..	1	3	*Nil*	*Nil*	3
3	..	·1	3	*Nil*	*Nil*	3
3	..	·05	*Nil*	3	*Nil*	3
3	..	·03	*Nil*	3	*Nil*	3
3	..	·01	*Nil*	3	*Nil*	3
3	..	·005	*Nil*	3	*Nil*	3
3	..	·003	*Nil*	3	*Nil*	3
3	..	·001	*Nil*	3	*Nil*	3
			·1		5	

	Sacchar.	Dulcite.	Adonite.	Inulin.	V. & P.	Indol.	Motility.	Name or Number.
1	+	−	+	−	+	−	−	Lactis ærogenes.
2	+	−	+	−	+	−	−	Do.
3	+	−	+	−	+	−	−	Do.
4	+	−	+	−	+	−	−	Do.
5	+	−	+	−	+	−	−	Do.
6	+	−	+	−	+	−	−	Do.
7	+	−	+	−	+	−	−	Do.
8	+	−	+	−	+	−	−	Do.
9	+	−	+	−	+	−	−	Do.
10	+	−	+	−	+	−	−	Do.

Note.—Steady widening of the line.

BOTTOM.—30-9-11.

			Glucose.		Lactose.	
1	..	20	1	*Nil*	1	*Nil*
2	..	10	2	*Nil*	2	*Nil*
3	..	5	3	*Nil*	3	*Nil*
3	..	1	3	*Nil*	3	*Nil*
3	..	·1	3	*Nil*	3	*Nil*
3	..	·05	3	*Nil*	1	2
3	..	·03	3	*Nil*	1	2
3	..	·01	2	1	*Nil*	3
3	..	·005	2	1	*Nil*	3
3	..	·003	1	2	*Nil*	3
3	..	·001	*Nil*	3	*Nil*	3
			·005		·1	

	Sacchar.	Dulcite.	Adonite.	Inulin.	V. & P.	Indol.	Motility.	Name or Number.
1	+	–	+	–	+	–	–	Lactis ærogenes.
2	+	–	+	–	+	–	–	Do.
3	+	–	+	–	+	–	–	Do.
4	+	–	+	–	+	–	–	Do.
5	+	–	+	–	+	–	–	Do.
6	+	–	+	–	+	–	–	Do.
8	+	+	+	–	+	–	–	67.
9	+	–	+	–	+	–	–	Lactis ærogenes.
10	+	–	+	–	+	–	–	Do.

SURFACE—5-10-11.

			Glucose.		Lactose.	
1	..	20	1	*Nil*	*Nil*	1
2	..	10	2	*Nil*	*Nil*	2
3	..	5	3	*Nil*	*Nil*	3
3	..	1	3	*Nil*	*Nil*	3
3	..	·1	3	*Nil*	*Nil*	3
3	..	·05	*Nil*	3	*Nil*	3
3	..	·03	*Nil*	3	*Nil*	3
3	..	·01	1	2	*Nil*	3
3	..	·005	*Nil*	3	*Nil*	3
3	..	·003	*Nil*	3	*Nil*	3
3	..	·001	*Nil*	3	*Nil*	3
				·1	*Nil* in 60	

	Sacchar.	Dulcite.	Adonite.	Inulin.	V. & P.	Indol.	Motility.	Name or Number.
1			*Nil*					
2								
3								
4								
6								
7								
8								
9								
10								

Note.—Total disappearance of lactose fermenters.

A description has already been given of certain experiments made in the large pond in the compound of our laboratory. It will be remembered that the whole of this tank was carefully polluted with a mixture of cowdung, human fæces, septic tank effluent, etc., and after the addition of sewage a series of samples were taken at various intervals. Unfortunately the results of these experiments are not so satisfactory as they might be for several reasons :—

(1) The fæcal material was obtained during the hot weather, when, as we have already pointed out, the number of varieties of organisms present was small.

(2) The water itself, of course, was not sterile and contained about one fæcal organism, usually of the very resistant type per cubic centimetre. In the light of these facts it is not reasonable to base any conclusion on these experiments, because it is not certain whether the organisms that survive were added with the fæces or are those that existed in the water previously. One experiment, however, is worthy of short notice.

Owing to the difficulty of obtaining a mixture of sewage which contained large numbers of coli communis, we added to the mixture of fæces 2 litres of a pure culture of bacillus Schäfferi. The sewage was added about 4 o'clock in the evening. Samples were taken at 7 in the next morning. In the first samples bacillus Schäfferi was found present in as small a quantity as 1 c.c. of the water taken from the surface and from the bottom of the tank. It was also isolated at 4 o'clock the same evening from a sample taken from the surface. The second day no bacilli was found on the surface but on the 3rd day they were also found. From this time forward bacillus Schäfferi could never be isolated from any surface or bottom sample. This is rather an important result, because in this case the organisms were laboratory cultures grown in broth, and we have already stated that, as a general rule, laboratory culture show a greater resistance to the action of inimical forces than do bacilli under strictly natural conditions, and yet, in spite of this fact, the coli communis disappeared more rapidly than many of the other bacilli added with the sewage.

CHAPTER IX.

THE EFFECTS OF SUNLIGHT CONTINUED: THE RESULTS OF ANALYSES OF THE WATER OF LAKES AND RESERVOIRS IN INDIA.

A THIRD source from which the action of sunlight and storage can be demonstrated, is by a study of the results of analyses obtained from the various impounding reservoirs, lakes, and storage tanks, taken at different times of the year, and considering these in relation with the meteorological conditions obtaining at the time. In the Madras Presidency 7 out of 18 water-works derive their supply from lakes; 2 others, namely, Kurnool and Cocanada, possess storage reservoirs, which for 9 months of the year are used as settling tanks only, holding about 6 or 9 days' supply; but which at the commencement of the hot weather are pumped full, and act as storage reservoirs for the town, containing as much as 2½ to 3 months' supply, for the period when the irrigation canals are empty. The results obtained in these 2 storage reservoirs have been included in these returns.

In order to save long descriptions Tables IX (*a*) and IX (*b*) have been compiled.

It is necessary to make a few remarks about the meteorological conditions obtaining in each quarter:—

(1) Quarter ending 30th September 1908. During these three months no rain had fallen in any of the towns given in Table IX (*a*), for many months prior to the taking of the samples, with the single exception of Ootacamund. All the storage reservoirs were extremely low; Adoni, Ootacamund and Vizagapatam were extremely short of water. Consequently the analyses give the results of waters that have been exposed to sunlight for a great length of time, in no case less than 6 months.

(2) Quarter ending 31st December 1908. In practically every town more or less rain fell during this quarter. In most particularly at Vellore, Ootacamund, Coonoor, Madras

TABLE IX (*a*).

Tabular Statement of Results of Analyses of Lake Waters.

Town supply.	QUARTER ENDING 30TH SEPTEMBER 1908.		QUARTER ENDING 31ST DECEMBER 1908.		QUARTER ENDING 31ST MARCH 1909.	
	Fæcal bacilli in ... c.c.	Organisms present and number.	Fæcal bacilli in ... c.c.	Organisms present and number.	Fæcal bacilli in ... c.c.	Organisms present and number.
Adoni	·1	10 Grunthal	·1	2 Coli communis, 5 Grunthal, 3 Coscoroba.	50	8 No. 73, 1 Cloacæ, 1 Coscoroba.
....	·1	4 Cloacæ, 1 Lactis ærogenes, 1 No. 73.	..	....	..	....
Coonoor	·01	8 Grunthal, 2 Coscoroba	5	1 Grunthal, 9 Coscoroba	1	10 No. 73.
Madras— 1 Red Hill ..	1	10 Cloacæ	5	9 Lactis ærogenes, 1 Coscoroba.	50	10 Lactis ærogenes.
2. Sholavaram ..	..	....	5	10 Lactis ærogenes ..	..	....
Ootacamund— 1. Dodabetta (Upper).	·01	3 Cloacæ, 4 Grunthal, 3 Coscoroba.	50	*Nil*	50	*Nil.*
2. Dodabetta (Lower).	·1	3 Lactis ærogenes, 5 No. 101, 1 Pneumoniæ.	1	4 Lactis ærogenes, 3 Cloacæ, 2 No. 67, 1 Neapolitanus.	1	7 Grunthal, 1 Acidi lactici, 2 Cloacæ.
3. Tiger Hill ..	5	10 Grunthal	·1	6 Cloacæ, 1 Grunthal, 2 No. 101.	50	*Nil.*
4. Marlimund ..	3	5 Coli communis, 5 Grunthal.	1	4 Cloacæ, 2 Coscoroba, 1 Grunthal, 1 Neapolitanus, 1 Acidi lactici, 1 No. 75.	1	7 Neapolitanus, 3 No. 73.
Vellore	·1	5 Cloacæ, 2 Neapolitanus 2 No. 73, 1 No. 67.	10	2 Grunthal, 2 No. 6, 6 Coscoroba.	10	10 No. 9.
Vizagapatam ..	1	10 Cloacæ	1	2 Cloacæ, 4 No. 67, 4 No. 75.	5	10 No. 67.
Kurnool*	1	10 Grunthal	·1	4 Cloacæ, 1 Grunthal, 4 No. 67, 1 Lactis ærogenes.†	5	8 Lactis ærogenes, 2 No. 101.
Cocanada*	1	3 Cloacæ 2 Grunthal, 1 No. 6.	10	10 Grunthal	1	2 Grunthal, 4 Coscoroba, 2 Lactis ærogenes, 1 No. 101.†
Approximate average.	·3 of a cc.		8 cc.		40 cc.	
....	Coli communis present in one instance.		Coli communis present in one instance.		Coli communis entirely absent.	

TABLE IX(*b*).

Tabular Statement showing percentage of Organisms in Lake Waters.

Name of organism.	QUARTER ENDING SEPTEMBER 30, 1908.		QUARTER ENDING DECEMBER 31, 1908.		QUARTER ENDING MARCH 31, 1909.	
	Actual.	Per cent.	Actual.	Per cent.	Actual.	Per cent.
Bacillus Cloacæ	35	31·8	19	16·8	3	2·2
,, Grunthal ..	49	44·5	21	18·6	9	10·0
,, Lactis ærogenes	4	3·6	27	24·0	20	22·2
,, Coscoroba ..	5	4·5	21	18·6	5	5·5
,, Coli communis ..	5	4·5	2	1·7	..	..
,, Neapolitanus ..	2	1·8	2	1·7	7	7·7
,, Pneumoniæ ..	1	·9	1	·8	..	..
,, Acidi lactici ..	..	..	..	..	1	1·1
,, Oxytocus Perniciosus	..	..	..	..	..	..
,, No. 73	3	2·7	6	5·3	21	23·3
,, No. 101 ..	5	4·5	2	1·7	3	3·3
,, No. 67	1	·9	10	8·8	10	11·1
,, No. 6	1	·9	2	1·7	..	..
,, No. 9	..	..	..	..	10	11·1
	111	100·0	113	100·0	89	100·0

and Adoni, extremely heavy rain fell, the totals being over 30 inches. The reservoirs were all replenished with water. In the case of Red Hills the rainfall lasted for a little over two months. During the year under report the rains were fairly normal, with the exception of Vizagapatam. Coconada also had deficient rain, but in this case the water-supply is not dependent directly on the monsoon, but on the irrigation canal.

(3) Quarter ending 31st March 1909. There was very little general rain during this quarter, but Madras, Ootacamund, Vellore, all received a considerable downpour, amounting to about 6 inches, within the first week of January 1909. After this the weather was fine.

There is, however, another point which must be mentioned, namely, that quite a large number of these reservoirs and lakes have supply channels in the shape of streams running into them. During the months of January and

February these were, for the most part, discharging. In March, April or May they usually dry up.

Of course, it is scarcely necessary to state that when rain is not actually falling in India, the sky is usually cloudless and the days are clear.

Let us now discuss the results of the bacteriological analysis of the water samples taken during these three quarters.

In the quarter ending 30th September 1908 it has already been stated that the lakes were extremely low. Therefore it may be assumed that the organisms present really represent those particular varieties which have succeeded in surviving during the hot and sunny months of the Indian spring and summer, a period of from 6 to 9 months.

A reference to Table IX (*a*) shows that fæcal organisms were relatively extremely common in the water of these lakes (as the figures in the narrow column demonstrate) : there is only one sample in the whole number that gives no fæcal bacilli in 5 c.c., *viz.*, Tiger Hill in Ootacamund ; in all the others fæcal bacilli are present in 1 c.c., ·1 c.c. or ·01 c.c.

In spite of the great power of the sun during the summer months, these organisms that are present have not been affected to any great extent, otherwise the water would be purer. Consequently it is certain that the organisms found at this time are indeed resistant ones. Both Tables IX (*a*) and IX (*b*) demonstrate the fact that 75 per cent. of the organisms present at this period of the year are cloacæ and the Grunthal-vesiculosus group. It will be observed, by looking at Table IX (*a*), that quite a fair proportion of the remaining 25 per cent. of organisms have been derived from the Ootacamund reservoirs where, as we have seen, rain had fallen. It may be objected that in the method of analysis which we adopted, the actual percentage prevalence of any particular organism is not a satisfactory indication of its real frequency in water-supplies. This objection does not apply when the average number of fæcal bacilli is between 1 and 10 per c.c. ; but it is a reasonable criticism when fæcal bacilli are scarce, such as 1 in 100, or 1 in 50 c.c. But even admitting this objection, it will be observed, that out of 12 different water-supplies examined, either cloacæ, or Grunthal, or both, were present in 11, and greatly predominated in no less than 8. The extraordinary numerical predominance of these organisms over all others, may be taken to be very strong evidence in favour of the truth of the statement, that these organisms are resistant to the action of sunlight.

Coli communis was isolated once in this quarter, from the Marlimund reservoir in Ootacamund. Inspection showed that the catchment area was contaminated to a considerable extent by wood-cutters, in the immediate neighbourhood of the lake, and that light rain was actually falling at the time when sample was taken. Coli communis was entirely absent from all other samples.

Second quarter.—A very marked difference has come over the results of the analyses of samples taken during the quarter ending 31st December 1908. First of all, there has been a great improvement in the average quality of the waters. An approximate average for this quarter is 1 fæcal organism in 8 c.c. of water. In no less than 6 out of 13 samples, fæcal bacilli were only present in 5 c.c. or more. But the most important change that is observed between the first and the second quarter is best shown by Table IX (*b*). The large falling off in the number of bacillus cloacæ and in the Grunthal-Vesiculosus group is apparent. The great increase in organisms, such as lactis ærogenes, coscoroba, should be observed. Bacillus cloacæ and Grunthal also persist, but only in very much the same proportion as they are found in all recently polluted surface waters. It will also be noticed, that, as compared with the samples of the previous quarter, a mixture of varieties of organisms is the general rule.

Coli communis were found in one case, namely, in the lake at Adoni. The explanation of this cannot be given satisfactorily, but it is probably due either to contamination at the edge, or to the fact that the supply stream was still flowing.

Third quarter.—The striking feature about the samples, taken during this quarter, is the great purity of the waters. The approximate average in the samples is 1 fæcal organism per 40 c.c. of the water. The reason for this is obvious, namely, that during this quarter, the large volume of water, which filled up the lakes, during the rains of October, November, December and the first week of January, has had ample time to get thoroughly well sunned, settled and stored. The consequence is, that, in no less than 4 instances out of 12, fæcal bacilli could only be found in more than 50 c.c. In no case were samples obtained which contained more than one fæcal organism per c.c. In comparing this quarter with the last the percentage prevalence of organisms presents little change. The organisms of medium powers of resistance still preponderate very largely. Lactis ærogenes and coscoroba are fairly plentiful. Cloacæ has fallen off to an extraordinary extent ; this is undoubtedly due to the tendency for these organisms to remain at the bottom of lakes, as has been demonstrated in Chapter VIII. Bacillus Grunthal is not common. It is obviously outnumbered by such organisms as lactis ærogenes and coscoroba. No coli communis were found in the analyses of this quarter.

The study of the results of the analyses obtained, side by side with the prevailing meteorological condition, demonstrates the extraordinary efficiency of these natural forces in getting rid of all but the most resistant elements of a pollution which is very great in amount at certain times of the year.

These remarks and tables of figures were compiled as long ago as the summer of 1908, and since that time we have had opportunities for correlating results that have been obtained in Madras and Bengal during more than two years. Table No. IX (*c*) gives the results of the prevalence of organ-

TABLE IX (*c*).

Percentage Prevalence of Organisms, Madras Lakes and Storage Tank, year 1910.

	Quarter ending March 31.	Quarter ending June 30.	Quarter ending Sept. 30.	Quarter ending Dec. 31.
Bacillus Grunthal-Vesiculosus	21·4	6·6	31·2	30
,, Coli communis Schäfferi ..	*Nil.*	2·2	2·4	*Nil.*
,, Lactis ærogenes ..	24·9	20·0	6·2	43·1
,, Cloacæ	36·2	5·5	6·2	15·7

isms during the four quarters of the year 1910 in lake waters in the Madras Presidency. During the year 1910 the rainfall was very equally divided over the whole of the year. In each quarter a certain amount of rain fell in most places; the consequence is, that although the table does not exactly tally with Table IX (*b*) which was made from figures obtained in 1908-09, it still shows in a marked degree the prevalence of certain bacilli at various times of the year, *viz.:* (1) the dry quarter of the year (with the exception of Kunoor and Ootacamund supplies) was from December to March, and in this quarter a very decided prevalence of cloacæ is observed. The Ootacamund and the Kunoor reservoirs, which have received rain, account for the prevalence of lactis ærogenes during this quarter. (2) By far the wettest portion of the year was the 2 quarters ending September and December, in every place heavy rain fell. In the first of these quarters (ending September), the waters were highly polluted and show mixtures of bacilli. There is no great prevalence of lactis ærogenes, nor of cloacæ. In the second of these quarters, although a great deal of rain fell, a certain amount of purification has gone on, and bacillus lactis ærogenes is the most common organism isolated. Bacillus cloacæ is, as compared with hot weather samples, a rare organism.

For the same year the samples obtained from rivers have been tabulated. Of course, it must be understood that rivers never show the stages of purification as clearly as lakes, because pollution may be added at all times along the banks. Table IX (*d*) gives the results for the Madras rivers. Again,

TABLE IX (*d*).

Percentage Prevalence of Organisms in Madras Rivers, year 1910.

	Jan.-June.	July-Dec.
Bacillus Grunthal-Vesiculosus ..	29·3	22·9
,, Coli communis and Schäfferi	5	8·7
,, Lactis ærogenes	15·0	25·8
,, Cloacæ	17·5	1·7

it will be observed that during the wet 6 months of the year lactis ærogenes is more prevalent than at any other time, and cloacæ are rare. During the dry 6 months of the year the reverse obtained, cloacæ is a very common organism, and lactis ærogenes is less frequently found.

The work that has been carried out in Bengal also bears out these statements very considerably. Unfortunately, however, there are no lakes or storage reservoirs in Bengal from which figures could be obtained. All the water supplies are obtained from rivers. Table IX (*e*) gives the results of the samples obtained in the large rivers in Bengal, exclusive of the Hughli, during the year 1911. In Table IX (*e*) the samples were divided into dry and wet weather samples. The rivers in this part of the country are, as compared with the Madras rivers, deeper, and muddier, and the population along the banks is usually greater. Consequently, one would not expect to find the same variation between the dry weather and wet weather conditions. Still in Bengal the following points must be noted :—

TABLE IX (*e*).

Percentage Prevalence of Organisms in Bengal Rivers, year 1911.

	Dry weather Dec.-June.	Wet weather July-Nov.
Bacillus Grunthal-Vesiculosus ..	41·3	23·0
,, Coli communis	3·1	13·5
,, Lactis ærogenes	7·5	19·2
.. Cloacæ	10·9	4·2

(1) In the dry weather samples lactis ærogenes is rarer than in the wet weather.

(2) In the dry weather samples cloacæ is commoner than in the wet weather ; and

(3) The vesiculosus and Grunthal group are extraordinarily common during the dry weather, as compared with the wet. It is not desirable to push this conclusion further

than this point. By far the most important point is that when the rain is prevalent lactis ærogenes is distinctly common.

It may, at this point, be stated that it is extremely difficult to show by figures giving percentage prevalence of organisms, the actual state of affairs in nature. The figures quoted above in a feeble way demonstrate the truth of our statements, but any one who works in the laboratory is simply astonished at the certainty with which one can predict what organisms will be found by knowing the local conditions at the time. For instance, even in the Bengal rivers where it is more difficult to show the natural prevalence of organisms, on account of the population on the banks, a sample of river water taken in the dry weather from a shallow part of the river will invariably contain large number of cloacæ. On the other hand, a river water that is clearing up after the polluting influence of a downpour of rain will certainly contain large numbers of lactis ærogenes. If the pollution amounts to a considerable quantity, such as is the case in a river Hughli, near Calcutta, it is not possible to show, at any rate by figures consisting of percentage prevalence of organisms, any change in the flora of the water due to variation in the seasons. Without forcing these figures in any way, we consider that it is legitimate to draw the following conclusions from all these results :—

(1) That resistant organisms do exist in nature, the commonest of these is bacillus cloacæ, which is always more prevalent in the dry weather than in the rains, when it is rare.

(2) During the time when the rains are common, lactis ærogenes is a very common organism isolated from rivers and lakes.

(3) During the dry weather this organism is rare. Hence it is probably not a resistant organism.

CHAPTER X.

THE POSITION OF CERTAIN FÆCAL ORGANISMS IN NATURE.

THE previous three chapters have shown that in the group of lactose fermenting organisms there is considerable difference between the various members of that group in the power which they possess to resist sunlight. At one time in the course of our work it was hoped that we might be able to place each organism in a graded list according to its power of resistance. We have come to the conclusion during the past three years that this is impossible, because amongst intestinal organisms generally, many appear to be about equal in this respect, or in other words, in Class II (the medium resistant group) the difference, if it exists, is so slight as to be of no practical use. It is, therefore, only such organisms as show very marked differences in their powers of resistance that are likely to be of any use to the water analyst. We shall now discuss these organisms and say anything about their position in nature that has been ascertained up to this time. We shall take susceptible organisms first.

Coli Communis of Escherich.

By far the most important of these susceptible organisms is the coli communis of Escherich, bacillus Schäfferi and certain other varieties (which only differ from each other by the positive or negative reactions of indol and motility). They are without doubt one of the most important group in human and animal fæces, and, as far as we know, this organism is found in the fæces of practically all animals. In tropical countries, however, it is distinctly a rare organism, but the frequency with which it is isolated varies very much; speaking generally it is much commoner in the rains than in the hot weather. This little group appears to be very susceptible to adverse influences. In our sunlight experiments we have never yet found that they survive many hours. In our work on naturally polluted lakes and in artificially polluted tanks (even though a laboratory culture is used) the same results were obtained. The organism is always associated with recent pollution. This has been shown by the use of the glucose and lactose broth. We have never found that true coli communis are present when the acid and gas line in the two media is widely separate. On one occasion we did find that this organism was present in the water of a lake which we knew was low and had been exposed to the sun for a period of 7 months without rain; but on inspecting the locality it was discovered that the catchment area had been largely contaminated by cattle (as it was the only available grazing in the district), and that buffaloes had been wallowing at the edge of the lake.

TABLE X (*a*).

Percentage number of typical B. coli, which did not ferment Saccharose.

1908-9. Month. Coals.	Raw Thames water. 232 samples. 1.	Thames filtered water. 4,951 samples. 2.	Raw Lee water. 232 samples. 3.	Lee filtered water. 409 samples. 4.	Raw New River water. 232 samples. 5.	New River filtered water. 1,353 samples. 6.
1908.						
April ..	85·7	86·2	85·3	1 out of 1	71·4	4 out of 5
May ..	65·6	70·0	74·6	1 ,, 2	75·5	4 ,, 5
June ..	77·2	59·7	60·7	43·7	62·7	5 ,, 7
July ..	53·3	54·2	63·5	54·5	63·1	50·0
August ..	46·4	50·8	62·1	2 out of 2	59·1	36·4
September	71·7	57·9	77·2	0 ,, 3	66·0	3 out of 9
October	70·8	57·0	78·2	4 ,, 8	74·6	53·8
November	70·1	70·2	84·7	2 ,, 3	72·9	75·0
December	80·6	81·5	88·5	83·3	70·7	81·1
1909.						
January ..	90·4	84·0	83·3	6 out of 7	67·8	80·9
February ..	90·4	86·8	84·5	1 ,, 1	73·9	2 out of 3
March ..	93·5	82·8	88·0	81·8	79·5	66·7
Average	74·4 (727 specimens)	72·0 (1,029 specimens)	77·3 (813 specimens)	63·6 (77 specimens)	69·6 (520 specimens)	67·0 (176 specimens)

Some of the figures in columns 4 and 6 are too few in number to be dealt with by this percentage method.

TABLE X (*a*)—*Contd.*

Percentage number of typical B. coli, which did not ferment Saccharose.

1910-11. Month. Coals.	Raw Thames water. 247 samples 1.	Thames filtered water. 4,861 samples 2.	Raw Lee water. 247 samples. 3.	Lee filtered water. 410 samples. 4.	Raw New River water. 247 samples. 5.	New River filtered water. 1,401 samples. 6.
1910.						
April ..	70·7	63·3	72·9	*Nil.*	63·9	1 out of 1
May ..	50·0	58·6	50·0	0 out of 2	65·5	3 ,, 7
June ..	50·0	34·5	62·5	3 ,, 7	63·0	30·0
July ..	42·2	38·6	44·0	1 ,, 6	38·3	25·0
August ..	57·4	48·7	58·1	2 ,, 7	46·4	46·6
September	55·5	35·7	40·0	41·6	48·1	33·3
October ..	78·6	51·5	71·2	1 out of 3	65·9	3 out of 8
November	69·2	69·9	74·7	2 ,, 2	69·5	74·5
December	80·0	72·4	81·1	3 ,, 4	76·0	55·1
1911.						
January ..	76·0	74·9	67·6	7 out of 8	62·5	48·3
February ..	77·9	81·6	86·0	2 ,, 2	67·7	5 out of 8
March ..	79·0	62·5	75·7	2 ,, 2	58·3	3 ,, 5
Average	68·1 (700 specimens)	63·3 (1,277 specimens)	66·7 (737 specimens)	50·9 (55 specimens)	60·3 (522 specimens)	56·3 (274 specimens)

Some of the figures in columns 4 and 6 are too few in number to be dealt with by this percentage method.

There is no evidence that coli communis multiplies when passed into water in nature; on the contrary there is a large amount of evidence that it dies out with considerable rapidity, particularly when it is subjected to the action of the sun rays. Certain figures collected from Dr. Houston's report also tend to confirm the statement that coli communis is a delicate organism. Two tables taken from his report demonstrate the fact that his "typical bacillus coli" is rarer in summer than it is in winter. Of course, Houston's "typical bacillus coli" includes some 8 or 10 organisms, but work on sewage and fæces at home show that amongst these 8 or 10, coli communis of Escherich is *probably* the commonest organism; consequently, although his figures cannot be quoted as absolute proof, it is highly probable that the greater rarity of typical coli communis in summer is due to the fact that coli communis of Escherich rapidly disappears in summer weather.

From a certain amount of work which has been carried out on polluted earth, coli communis has been found on one occasion to remain alive in ordinary damp ground for a period of 97 days.

Oxytocus Perniciosus and Bacillus No. 98.

These organisms have the most remarkable position in nature. It is not by any means certain that this is a true fæcal organism, but there is no doubt that it is fairly frequently found in fæces of man and animals at certain times of the year. In India, both in Madras, Bengal, and in the United Provinces, these organisms have been found in rivers, lakes and well waters at one time or another during heavy monsoon weather. They are nearly always found in water with every indication of very recent pollution. The samples obtained show that in about 90 per cent. the lactose fermenters were present in ·01 of cubic centimetre; the total colony counts are extremely high, usually about 2,000 or 3,000 per c.c.; in about 50 per cent. coli communis was also associated with oxytocus; and in the few samples where glucose and lactose broth have both been used, the acid and gas line is practically identical in the two media. Consequently, there is every reason for supposing that this little group of organisms is usually found in connection with dangerous and recent pollution. Very occasionally in a comparatively good water one of these organisms is isolated. This occurred once or twice in certain rivers in Madras. In our work on the Red Hills Lake in Madras in 1909 in Chapter VIII it was shown clearly that this organism disappears with very great rapidity. It is never found in dry and hot weather, either in the fæces of man or animals, or in water-supplies. A careful and exhaustive search was made, using inulin broth, in order to try to isolate this bacillus; on every occasion this proved unsuccessful, except during the monsoon weather when in some years it was quite common. The great rarity of these organisms at most times of the year renders their study extremely difficult, but the little we do know about them is based on sound evidence collected from Madras, Bengal, and the United Provinces. The facts may be recapitulated: in the great majority of cases it appears in fæces during heavy monsoon weather, and at the same time it is to be isolated from water-supplies; it is practically never found except when the water is very grossly polluted with fresh material, and it disappears with very great rapidity. It has not yet been definitely ascertained whether the organism is a true inhabitant of intestinal tracts of man and animals, or is just a passing guest.

Bacillus Lactis Ærogenes.

Bacillus Lactis Ærogenes occupies a very extraordinary position in nature. First of all, it must be stated that the organism is rare in fæces. One or two per cent. of all organisms isolated is about what one would expect. Up to the present no sudden prevalence of this organism has ever been found in fæces, as has been recorded of certain other organisms. In grossly and recently polluted water lactis ærogenes is a rare organism, but usually within a period of about 5 to 15 days after pollution in lakes the organism becomes extremely common.

Particular attention is directed to Chapter VIII where a study of the Red Hills Lake was discussed. There it has been shown on several occasions after pollution of a gross character, lactis ærogenes is invariably very common some days after the pollution was added. Besides this the figures given in Chapter IX are in themselves absolutely indisputable. In rivers the period of time when rain is common is characterised by an enormous increase in the number of lactis ærogenes, and yet we are perfectly certain that the organism is rare in fæces. Furthermore, the bacillus is not a very resistant one, because, as time goes on, if no further pollution is added, lactis ærogenes disappears and gives place to the true resistant varieties. The figures already given are convincing as to the truth of this statement. There can be no doubt that at a certain stage in natural purification, lactis ærogenes multiplies in water. It has been proved in our laboratory that it does not do so in laboratory experiment; that is to say, if water is contaminated with fæces and kept in a bottle no increase of lactis ærogenes ever takes place. Again, our sunlight experiments, although they demonstrate the fact that the bacillus is uncommon when the emulsion is first made and usually appears about half way down the experiment, and disappears before the very resistant organism, still there is nothing to show that multiplication of the organism is usually present. In large volumes of water such as rivers and lakes, the increase in numbers is undoubted.

From the point of view of the water analyst, this is of very great importance. When lactis ærogenes is very plentiful it may be fairly certain that the true fæcal arrangement of the organisms in the pollution has been altered. Additional evidence of this fact could be obtained by the use of both glucose and lactose broth, when it would be found that there had been a considerable decrease in the number of lactose fermenters, with comparatively small decrease in the glucose fermenters. Examples of this are met with in the second series of experiments carried out in the Red Hills Lake in October 1911, some results of which are given.

This subject certainly requires more study, but at the same time it would appear that bacillus P. and lactis ærogenes largely resemble each other in their position in nature. Both are rare in fæces; at a certain stage both increase very rapidly under natural conditions in water, but whereas bacillus P. persists for a very long period without diminishing its numbers, lactis ærogenes disappears, giving place to the very resistant type of lactose fermenting bacteria. From the work given in Chapter VIII it would appear that lactis ærogenes is not very common at the bottom of lakes, but is more prevalent nearer the surface and in the middle.

The organism is not frequently met with in our work on highly polluted earth (this work is not described in detail in this volume).

Bacillus Cloacæ.

We will now consider the resistant organisms. Probably the most interesting member of this class is bacillus cloacæ. This organism is one which shows a very great variation in its numbers in the fæces of man and animals at different times of the year; it may constitute as much as 15 to 20 per cent. of all organisms present at one time, or it may be as low as 1 or 2 per cent., or be entirely absent at another. It would appear from our work that bacillus cloacæ is commoner in fæces in Madras than it is in Bengal, but this requires further investigation. The period of prevalence in fæces is usually about May, June and July, in the height of the hot weather. Not only is the organism extremely prevalent in fæces, but at this period of the year it is extremely common in lakes and, to a less extent, in rivers in Madras. In Bengal we have no lakes worthy of the name, and therefore it is impossible to say whether it is so common in this part of the country, but it has been shown to be fairly prevalent at the bottom of tanks, and less so in rivers. The figures given in Chapter IX demonstrate the periodical increase in the number of cloacæ in water-supplies contemporaneous with the dry and hot weather. Indeed the figures themselves do not really indicate how extraordinarily prevalent this organism is at certain times of the year. Lakes, many rivers, and even well waters contain practically nothing else when they are getting very low. The work carried out on the Red Hills Lake, described in Chapter VIII, shows that even when large volumes of highly polluted water pass into the lake, the bacillus cloacæ settles to the bottom and is by far the commonest organism in this position. Probably they slowly multiply; and as the water gradually falls this organism appears to be the only one of all the fæcal organisms present. The sunlight experiments show that it is amongst the most resistant of lactose fermenters. It usually appears late in the experiment, and in quite a fair number, it is the last organism to survive. The bacillus is also extremely common in damp river sand; in the work carried out on polluted earth derived from "trenching grounds," in practically every case *when the pollution is over a year and a half or 2 years old, bacillus cloacæ was the only coliform organism that could be isolated.* The presence of this organism in large numbers in any water usually, but not invariably, indicates that it comes from a lake that is getting low or a river that is drying up. It has already been pointed out that during the hot weather the number of bacillus cloacæ appears to increase in the fæces of human beings and animals, and in water-supplies simultaneously ; the fact that there is no rain falling shows that the increase in the water-supplies is not due to the increase in fæces. Were this the case, the type of pollution present in the water-supplies would have all the appearances of recent pollution, *viz.*, usual fæcal arrangement of bacilli, and the closeness of the acid and gas line in glucose and lactose ; but this is not noticed at this time of year, so that the great prevalence of cloacæ in water is not due to this cause. Inspection of the lakes in question shows that pollution does not take place when no rain is falling.

The Vesiculosus and Grunthal Group.

We have already shown that bacillus cloacæ is essentially an organism which belongs to the bottom of lakes and to river sand bacillus ; one organism of the Grunthal-vesiculosus group is also extremely resistant in a somewhat different way. In all probability bacillus vesiculosus is the resistant member of this little group, but as all species give negative results in all the sugars, it is extremely difficult in practice to decide between the various species. After considerable pollution has taken place, and the lactis ærogenes bacilli have begun to disappear, usually the only organism that can be obtained from the surface of a lake is either bacillus Grunthal or bacillus vesiculosus. The bacillus is particularly common in surface samples obtained from settling tanks, storage reservoirs and rivers in the hot weather ; it usually is common after the disappearance of the lactis ærogenes and before the water has got low enough to reach the cloacæ. It is the common bacillus of a well sunned *full* reservoir. The sunlight experiments also demonstrate the fact that it is in reality a resistant organism, it not unfrequently persisted to the end in these.

Unfortunately, the organism itself is a common one in fæces, so that it may still be present in considerable numbers in any sample, without really representing any very considerable departure from the true fæcal arrangement, but the figures in Chapter IX show that on the whole it is not so common in wet weather as it is in dry. It is an organism whose presence may give much valuable information, but on the other hand a good deal of care is required in drawing conclusions from results containing it.

The following brief remarks concerning other common fæcal organisms are given. As regards their powers of resistance to sunlight they all belong to class II. We do not consider that it is possible to divide these organisms further as they appear to be very similar in their powers of surviving adverse influence.

Acidi Lactici (*No.* 1 *or No.* 2).

This organism is usually found in small numbers in the fæces of man and cattle. Its presence is very constant. It has a slight tendency to predominate numerically in the fæces of man. It is also found in small numbers in water samples, particularly those taken from rivers and lakes. In contaminated earth it is fairly common, and it has on one occasion been isolated from ground, known to have been contaminated many months previous to taking the sample. Its entire absence from the water-supplies in the hot weather shows it does not rank with class III. Certain analyses in Chapter VIII, however, show that it survives till quite late in the exposure. A series of experiments, not yet complete, seem to show that it is very easily killed by desiccation.

Levans No. 3.—This organism is extremely rare, only having been met with on two or three occasions. Once it was isolated from human fæces, and once from Ootacamund sewage. No further details can be given.

Bacillus No. 6.—Is a distinctly rare organism. It was isolated once from human fæces. It is very much more common in water, and the sunlight experiments showed that it was not infrequently met with. It has been found in both cowdung and human fæces.

Bacillus No. 9.—This organism very much resembles acidi lactici, with the exception that it gives Vosges and Proskauer's reaction. It has only been isolated once from fæces, namely, from cowdung. In the month of February it was found to be very common in the water-supplies of Vellore and Gudiyattam, and rarer in a few others. The prevalence of this organism in several places tends to show that it is a separate species, and that it is not an odd specimen of acid lactici, which (according to some authorities) has acquired the power of giving Vosges and Proskauer's reaction. Nothing can be stated as regards its power of resisting the action of sunlight.

Bacillus No. 33.—This bacillus is similar to coli communis except that it ferments adonit. It is a moderately rare organism, appears to be more common in human fæces than in cowdung. It is occasionally found in water samples. For a long time we looked upon it as being confined to human fæces only, but extended work proved this to be incorrect. It possesses the power of resisting sunlight to a moderate degree and belongs to class II.

Bacillus Pneumoniæ and Rhinoscleroma No. 68.—This organism is usually found in small numbers in human fæces, cowdung and in water-supplies.

Bacillus No. 67.—Is common in samples of fæces of both human beings and cattle. It is also met with fairly commonly in water-supplies.

Neapolitanus No. 72, *and Bacillus No.* 74.—This is a fairly common organism and remarkably constant at all times of the year and in all samples of human fæces and cowdung. It is also constantly prevalent in water-supplies.

Bacillus Nos. 73 *and* 75.—This is an organism that has been isolated from cowdung and human fæces and is fairly prevalent in water-supplies. It has also been isolated from the trenching ground earth a considerable time after pollution had taken place. It is undoubtedly a resistant organism, but further work is necessary as to which class it should go into. At present it is temporarily placed in class III, amongst the resistant organisms. The fact that it is somewhat rare renders it difficult to put into its proper place without more work.

Bacillus Nos. 100 *and* 101.—This is an organism about which we were at first inclined to be sceptical, in spite of the fact that several bacteriologists claim to have isolated it. It is nearly always found in mixtures with lactis ærogenes, and the difference between these two bacteria is mainly the presence or absence of Vosges and Proskauer's reaction. There is no doubt that it is a separate organism. It is met with in the fæces of both man and cattle, but more frequently in the latter. At the same time there is also no doubt that attenuated specimens of lactis ærogenes lose their power of giving the Vosges and Proskauer's reaction; so when both organisms are found together in a water we look on it as probably being lactis ærogenes.

Coscoroba or No. 106.—This organism, next to the Grunthal group, has been found by us to be the commonest in the fæces of man and domestic animals. As the analyses show, its frequency varies greatly at different times of the year. It is very common in water, particularly in such water as we are certain is derived from washing the surface of a polluted ground. It has not been found common in the samples of earth we have taken. It is doubtful if this bacterium should not be in class III.

Bacillus P.—Although organism P. is not a lactose fermenter, it must be considered along with the others. First of all the reason we have named it organism P. is because at one time we thought it was almost identical with the ordinary sewage Proteus, but on further investigation it is found to differ very considerably from the classical organisms which have been described by many bacteriologists. On finding that the organism was not sewage Proteus, we still wrote it in our laboratory returns as bacillus P.

There is no doubt, whatever, that this is by far the commonest organism of fæcal origin in ordinary surface waters. As already pointed out it is quite rare in fresh fæces, but there is no doubt that it multiplies in water in spite of the sun. We have never yet discovered a well sunned sample of surface water from which this organism may not be isolated, and if the pollution is old the organism is extraordinarily common. The following appear to be the various fermentative reactions of this bacillus :—

The organism does not ferment lactose, but after it has been subcultured for some time in the laboratory a very faint trace of acid, though no gas, is sometimes seen. This is rather interesting, and at one time we thought it was remotely possible that this bacillus was really a true lactose fermenter which had lost its power of fermenting this sugar owing to exposure to the sun. Further experiments and consideration, however, have negatived this view. Acid and gas are produced in large amount in both glucose and saccharose. Neither acid nor gas is produced in mannite, dulcit, adonit and inulin. The organism does not liquefy gelatine within 10 days, nor does it give the indol reaction. The Vosges and Proskauer's reaction is given very strongly. In litmus milk there is a very slight acidity at the top of the tube in some cases, but no clot is produced. The bacillus is very actively motile and is gram positive. The bacillus has all the usual appearances of coli communis under the microscope, but gives a rather opaque white colony on neutral red lactose agar.

These are the characteristics of bacillus P. The other important point is as already stated, its ubiquity.

Let us briefly recapitulate what are the various stages that occur in the self-purification of natural water, such as a lake after it has been heavily polluted by surface washings. We start with a gross recent pollution the indications of which as we have already seen are :—

(1) The truly fæcal nature of the mixture of organisms isolated from a sample.

(2) The presence of susceptible organisms, particularly coli communis, oxytocus perniciosus, etc.

(3) Identically the same result is obtained in a glucose and lactose broth ; acid and gas line the same in both.

(4) The great rareness of white colonies on solid medium obtained from a glucose broth.

(5) The extraordinary difficulty of isolating the bacillus P at this period.

(6) The rarity of lactis ærogenes.

(7) The fact that resistant organisms, such as cloacæ and vesiculosus are not prevalent in large numbers, but may be isolated in smaller numbers in a fæces-like mixture.

(8) The high figures of the total colony count.

(9) Fæcal organisms, both lactose and glucose fermenters, present in ·01 of a c.c. or less.

(10) Possibly streptococci may be isolated from 20 c.c. of the water.

These are the various points noticed in a gross recent pollution. The first thing that will occur during the process of self-purification will be a considerable decrease in the total number of fæcal organisms within the first 3 days, so that they can only be isolated in ·1 or 1 c.c. Even at this period there would be a tendency for the acid and gas line in glucose and lactose to separate. Within the second week the following changes would have taken place :—

(1) The number of *varieties* of organisms isolated in 10 colonies would have decreased.

(2) There would be a marked tendency for lactis ærogenes to predominate.

(3) The sample taken from the bottom of a lake would probably show an increased prevalence of bacillus cloacæ.

(4) The acid and gas line in glucose and lactose would have separated, glucose + lactose – would probably be present in ·05—·1 of a c.c. in the water, lactose fermenters being present in 1 c.c. or 5 c.c. on the surface, possibly in much less.

(5) Bacillus P would now be very common and easily isolated, no other member of the class glucose + lactose – would be found.

As time goes on the next marked change that would be noted would be the tendency of lactis ærogenes to disappear from surface and middle samples, and to be replaced by bacillus vesiculosus. Within the first four weeks, unless the water was run off, the surface sample would be extremely pure. It would probably contain no lactose fermenters in 60 c.c. and no glucose fermenters in 1 c.c. Supposing now the water of the lake is used to supply a town, the clear surface water would be drawn off, and the whole appearance of the water would gradually become slightly hazy and muddy due to silt. At this period (say 3 months after pollution) the water will show either pure cultures of bacillus vesiculosus or cloacæ, or a mixture of the two. The bacilli will also be numerous, very likely be present in ·1 c.c. Bacillus P will also be extremely numerous, probably will be present in ·01 c.c. Beyond this point practically no change would take place.

This appears to be bacteriology of the self-purification of a lake that has been badly contaminated. It is fundamentally the same in rivers, particularly if they are slow and shallow, but it is not easy to demonstrate these various stages in deep rivers containing a certain amount of suspended solids in the water, where a quantity of pollution is constantly being added along the banks.

CHAPTER XI.

THE APPLICATION OF THE FOREGOING RESEARCH TO WATER ANALYSIS.

IT will, I think, be admitted that a thorough knowledge of the subject of the self-purification of water is likely to be of great value to the water analyst, particularly as we have already shown, there are certain well defined stages in the process. There are, however, several points which should not be overlooked in dealing with water analysis as a whole.

In the first place, it is pretty well recognised that to send a sample of water to an analyst without giving him any clue as to where the water is taken from, and to omit all details regarding the obvious chances of pollution, the amount of rainfall, etc., is a futile thing to do. Those who are inclined to scoff at water analysts frequently tell us that an inspection of a supply is of much more value than an analysis. In some respect this is undoubtedly correct. Anybody who has been in the habit of making sanitary inspections can with a very reasonable amount of certainty give an opinion as to the *likelihood* of dangerous pollution being present in any particular source of supply. The following is an instructive example. Two samples of water were sent for opinion as to which of the two sources was likely to be the better supply for some troops engaged in manœuvres. Sample No. 1 came from a stream running down a mountain side and was reported to contain a very large number of fæcal organism. Sample No. 2 came from a well situated near a village. The analyst remarked that the latter water was a pretty good one, and contained about 1 fæcal organism in 4 or 5 c.c., consequently he recommended the well to be used as a source of supply to the troops. The Medical Officer with the troops, who received this information, ignored it, because the well was situated in a village where cholera was not infrequently present, whilst the stream came from the side of a hill on which a fair number of cattle grazed during the day, but was practically free from all chances of human, and therefore dangerous, contamination. In this case the Medical Officer on the spot was undoubtedly right. When dealing with raw waters an analysis without inspection is of very little value indeed from a purely practical point of view.

As a general rule, samples in India roughly divide themselves into two classes. The first of these are raw waters taken from rivers, lakes, etc., on which the analyst is asked to pass an opinion as to whether they are fit for human consumption at any given time, or how much purification is necessary to make them so. The second variety of samples are usually filtered samples from some town works, on which one is required to report whether the filtered water is a good one, and fit for human consumption, and whether the filters are working satisfactorily, or whether leaks or other defects appear to be present in the installation as a whole. It is in this class of work that analysis is of such vital importance, and inspection alone is not sufficient evidence that hidden defects do not exist. In tropical countries the former of these two kinds of samples is much commoner than in Europe, because in the West raw river or lake waters are not used as a source of drinking supply, they are usually so polluted that they are useless without some artificial purification. Again, in the tropics, one is not infrequently brought face to face with the question. How much and what kind of purification is necessary in order to render such and such a source fit for human consumption? Now, it is perfectly obvious that unless we are thoroughly acquainted with the condition of the raw water at all times of the year, a great deal of unnecessary expenses might be incurred in plant to purify water that was in reality safe and only required clearing.

The splendid work recently done by Houston on the subject of Storage has undoubtedly greatly modified opinions on the subject of water purification. He has shown that a very bad sewage-polluted water can be rendered safe by simply storing it in a reservoir for an appropriate length of time. We have gone one step further in this direction, namely, by a study of the process of self-purification, certain definite bacteriological stages in the process have been discovered; so that, although it may be impossible to estimate by any ordinary means exactly how far the natural purifying forces have affected the water, if any sample shews distinct indication of being in any of these stages, we assume that a certain degree of departure from the original fæcal arrangement has been reached. Consequently, we are nowadays not so much interested in the number of organisms per cubic centimetre, or even the number of fæcal organism per c.c., as we are in the answer to the larger question—"Has this particular sample passed the safety change?" We have, we think, demonstrated that the actual number of organisms present, fæcal or otherwise, is subject to considerable variation under natural conditions, and is not a satisfactory guide to the real condition of the water. Thus, supposing a sample of water showing fæcal organisms in 10 c.c., showed also that the acid and gas line in glucose and in lactose broth was the same, and that the varieties isolated belong to the susceptible group, one would be perfectly justified in being highly suspicious of such a sample. On the other hand, a sample of water containing lactose fermenting organisms in ·5 c.c., glucose fermenting organisms being present in ·01 c.c., and the lactose fermenting organisms consisting of practically a pure culture of bacillus cloacæ, one would be prepared to say that such a sample was absolutely safe from the point of view of the possibility of conveying disease. In spite of the *number* of fæcal organisms present, an analyst in India would decide in his own mind that the sample was taken from a lake that was probably nearly dry, the water of which had been exposed for 6 or 8 months to a tropical sun, the bacilli being the residue of a pollution months old. Samples of this kind are quite common in the East. These examples demonstrate the utter futility of a hard-and-fast numerical standard of purity of waters. Each separate sample should be considered on its own merits, taking into consideration all the information that it is possible to obtain. The more one knows about the natural processes of self-purification of water, the more certain one is that it is necessary to study *the kind of organisms* rather than their number in order to arrive at a satisfactory conclusion.

It is necessary at this point to give a little more detail in the use of the classification of organisms according to their power to resist the action of sunlight. When dealing with a sample, one of the first points that an analyst should consider is, how much exposure has this water had to sunlight? and secondly, what are the chances of pollution? It will usually be possible to answer these from the information sent with the sample. Having made up his mind on this point he will proceed with the analysis, and ascertain what he is actually to state of the sample.

The number of all organisms that occur on agar at 37° C. will be ascertained; the number of fæcal organism per c.c.; the kind of lactose fermenting organisms present, and the relation between the glucose + lactose +, and the glucose + lactose −. We have already discussed most of these points in detail in other parts of this work, consequently little need be said on any of them here.

It is obvious that any sample showing a very large total count, such as 3,000, 4,000, or 5,000 organisms which grow on agar at 37° C. will be suggestive of pollution, but without the other parts of the analysis the fact is of no very great value in itself. Water organisms are often extremely common in comparatively unpolluted surface waters in this country. Furthermore, there is a class of spore-bearing organisms, more or less allied to bacillus mesentericus, which are extraordinarily prevalent in the rivers in Bengal, though less so in Madras. These overspread the whole of the surface of the plate and render counting impossible.

As regards the actual number of fæcal organisms present per c.c. this, as we have already seen, does not give any very accurate information about the time of pollution. Of course, it is perfectly obvious that in water containing no lactose fermenting fæcal organism in 60 c.c. (samples of this kind are very frequently found in lakes about 2 to 4 weeks after they have been replenished by heavy rain), the water is relatively safe and pure, but their presence in small quantities, such as 1 to 10 per c.c., does not necessarily indicate that the pollution is either recent or dangerous.

Concerning the subject of acid and gas line in glucose and lactose broth we have said practically all that is necessary in Chapter VI. The conclusion we arrived at was that in all cases when the acid and gas line is similar in broth media the pollution is recent, that is to say, the fæcal arrangement of the organisms is still present. The wider the divergence, the safer the water, and the older the pollution.

Turning now to the subject of the three classes of lactose fermenting organisms, *viz.* (class 1) the susceptible, (class 2) the intermediate, and (class 3) the resistant organisms, the following points may be mentioned :—

In his own mind the analyst will subdivide the samples into (1) those which contain susceptible organisms, (2) those which are made up almost entirely of class 3, or the resistant organisms, and (3) those which contain a mixture of the two or more classes, but in which class 2 predominate. Let us deal with each of these separately.

(1) A sample containing coli communis and other members of the susceptible group will present very little difficulty in arriving at a conclusion. Coli communis represents undesirable pollution, because it rapidly dies out from water, therefore its presence is suspicious. The following points will also be observed in samples of this nature :— (*i*) Probably 3, 4 or 5 different varieties of bacilli in the 10 colonies sub-cultured will be observed, the large number of varieties suggest the true fæcal arrangement. (*ii*) Nearness of acid and gas line in glucose and lactose broth. (*iii*) Possibility of finding streptococci in water. (*iv*) Scarcity of bacillus lactis ærogenes.

With this amount of confirmatory evidence one will have no difficulty in coming to the conclusion that the pollution is recent, and therefore dangerous. In tropical waters, samples containing susceptible organisms are distinctly rare, consequently, one has frequently to rely on the confirmatory evidence alone in giving an opinion. In rivers, however, where contamination along the bank is common they are more frequently met with, and always indicate dangerous pollution.

(2) It is probably not realised by those unacquainted with tropical conditions how extremely commonly samples of water containing resistant organisms *alone* are met with. It appears to us doubtful whether such samples are ever found in Europe. Consequently whenever such samples are found they may be looked upon as safe, as far as their chances of spreading disease is concerned, in spite of the fact that both lactose fermenting and glucose fermenting organisms are extremely common in the samples. Other confirmatory evidence as to the correctness of this dictum will be found in that the acid and gas line in glucose and lactose broth is widely separate; that the lactose fermenting organisms do not show a large number of varieties, but are usually a pure culture of either bacillus vesiculosus or bacillus cloacæ.

(3) Those containing a mixture of two or more classes; the chief difficulty that will be met with by an analyst using this method will be to arrive at an opinion from samples showing a mixture of classes 2 and 3. It is when dealing with samples of this kind that it is absolutely necessary to know particulars concerning where the samples come from, the amount of rainfall, etc. It must also be borne in mind that in the hot weather the number of varieties of organisms in fæces generally seems to fall off. Consequently one must not expect too many varieties even with a recent contamination. Another difficulty is the fact that such organisms as bacillus vesiculosus are extremely common in perfectly fresh fæces. Consequently too great stress must not be placed on their presence in samples of water containing this mixture of classes 2 and 3. In attempting to arrive at a conclusion one must be guided by (*i*) the actual number of fæcal organisms present in the sample; (*ii*) the results obtained from the acid and gas line in the glucose and lactose broth; (*iii*) the presence or absence of a large number of bacillus lactis ærogenes. A water containing a large number of this organism shows that it has reached the stage of "lactis ærogenes prevalence," and has departed thus far from the original fæcal arrangement. This fact taken with the acid and gas line in glucose and lactose will be found to give the best indication as to the date of pollution, but it is admit

ted that samples do occur in which it is extraordinarily difficult to be sure that a correct interpretation has been arrived at.

Although ground waters are not discussed in this little book, we consider it advisable to say just a few words on the subject.

Probably the greatest objection to the method that we propose is, we do not know how far it applies to waters from such sources. The question is much more complicated than those met in dealing with surface waters, the difficulties being (*i*) to estimate the importance and significance of organisms that remain alive in the soil for long periods, and (*ii*) to be quite certain when a pollution is caused by surface contamination, washed downwards through cracks or through a porous soil. In dealing with well waters the great point to look to is the quantity of fæcal pollution, and the results obtained in glucose and lactose broth. Of course, when coli communis is itself present with a mixture of other organisms, there can be little doubt that contamination of a dangerous nature has taken place. But when, as is so often the case, most of the bacilli present consist of a mixture of class 2 with probably a few of class 3, how far is it safe to assume that this type of pollution is not serious? It would not be wise to place much importance on the power of resistance of the organisms present, for in well waters we are not dealing with the resistant majority, but with the whole lot resistant and susceptible alike. Not infrequently, a well water, in normal hot weather conditions, containing only one organism, and that a resistant one, will, with comparatively slight meteorological changes, suddenly be found to contain a very large number of coli communis. We are at present in absolute ignorance both as to where these organisms come from (for they certainly do not come from recent surface pollution), and also as to what is their precise significance. They cannot be looked upon as altogether harmless, because, supposing the theory is correct that these organisms remain alive in the damper layers of soil, there is no certainty that the organisms of enteric and cholera may not do so as well. Consequently one is bound to regard their presence with great suspicion. On the other hand, in what may be termed the hot weather condition of wells and springs, bacillus cloacæ is nearly always the only organism present, and, knowing as we do, that this bacillus is gifted with great longevity in soil, no very great significance can apply to the presence of these organisms, provided that the number is not too large. We can only therefore advise as follows: (1) look with grave suspicion on all fæcal bacilli in well water; (2) be guided more by the quantity of fæcal organisms per c.c. than by the kind of organisms; and (3) on no account ever give an opinion without an inspection of, or a report on the surroundings by a Medical Officer. It should be borne in mind that extremely valuable information, as to the possibility of the entrance of the fæcal contamination, can be obtained by taking samples from the wells shortly after heavy rain; and, in case the well water is the sole drinking supply of a community, such as a jail or school, a series of analyses of samples taken under different meteorological conditions should always be made in doubtful cases, before giving a definite opinion.

Of course, these remarks apply mainly to surface wells. Deep wells are comparatively rare in India. There is no doubt that the surface well is an extremely bad source of water-supply, and that it is probably responsible for a large proportion of the water-borne disease in this country. Unfortunately in many places it is the only supply available.

This brings us back to the question already referred to, *viz.*, when may a badly polluted water be considered to have passed "the safety change"? The question is obviously one of very great importance, and one that is surrounded by very many difficulties. In the first place, it assumes a fact which is probably correct, *viz.*, that all the important pathogenic intestinal organisms are in the most uncongenial environment when placed in water, and they die out with great rapidity when exposed to the action of sun, etc. For the truth of this statement there is now a considerable amount of evidence. The work of Houston on the viability of cholera and enteric organisms in water kept in a laboratory confirms this opinion. Horrock's investigations on the symbiosis of Eberth's bacillus and coli communis also supports the contention. It should have, however, to be pointed out that results almost identical with those obtained by Houston can be obtained with fresh ordinary non-pathogenic fæcal organisms under the same conditions, so that these experiments do not necessarily prove that pathogenic organisms are more likely to die out than are non-pathogenic. From a very considerable study of the whole subject of pollution of water we are distinctly of opinion that in India 90 per cent. of all intestinal organisms die in water probably within the first 48 hours, but it is the remaining 10 per cent.—the resistant residuum—which undergo the various changes which we have already discussed. Assuming therefore that pathogenic organisms disappear very early in the process of natural purification, it may be taken as certain that as soon as any very decided departure from the original fæcal arrangement is apparent, the probabilities are that the water is safe. Now in really badly contaminated water the following points are always noticed :—

(1) Presence of streptococci in 20 c.c.
(2) Acid and gas line in glucose and lactose broth is the same.
(3) Usually, but not invariably, the presence of some member of the susceptible group.
(4) Absence, or great rarity of, the organism lactis ærogenes.

From what has gone before, it may be stated that the water has probably passed the safety change when *all* the following conditions are observed (1) no streptococci are found in 20 c.c. ; (2) the acid and gas line in glucose and lactose show a considerable widening ; (3) when no organisms in class 1 are to be found in 20 c.c. ; and (4) when there is a predominance of lactis ærogenes.

As a result of our experience at present, we venture to state that this usually takes place, in a clear water, in sunny weather, within one week, but this figure is given with very great reserve, because more work is necessary, and because during monsoon conditions this statement probably does not hold good.

In spite of what we have said about numerical standards of purity of water, it is necessary to give some sort of outline of the results of our experience, so the following tentative standards are given for tropical countries generally, and India in particular. But it must be distinctly understood that they are not hard-and-fast rules.

Let us first dispose of filtered samples. All samples of water that passes through filters should be free from organisms generally. From a study of filters in India, it is perfectly possible to get a water which should contain no lactose fermenting fæcal organism in 100 c.c. and only about 50 to 100 colonies on agar. Consequently any divergence from this rule should be carefully investigated. The commonest causes of failure of filters in this country are :—

(1) A very highly polluted water being put into the filters direct without previous purification ;
(2) Unsuitable nature of the sand ;
(3) Too frequent scraping ;
(4) Wrong design of the filters themselves, and bad manipulation ; and
(5) Presence of frogs and fish in the clean water reservoir.

When any sample of filtered water is unsatisfactory, that is to say, shows fæcal organism of any kind, present in large numbers, careful investigation should be made as to which of these causes is the one operating in any given installation.

RAW WATERS—LAKE WATERS.

A *good* lake water should contain less than 100 colonies per c.c. (on agar at 37°C.).

No lactose fermenters in 50 c.c.

No lactose fermenting organism of class 1.

Glucose fermenting organisms may be present in 1 or 5 c.c.

A *fair* or usable lake water should not contain more than 400 organisms (on agar at 37°C.).

Lactose fermenters should not be present in less than 1 c.c.

Glucose fermenters may be present in ·1 c.c.

No organism of class 1 should be present in 20 c.c.

Lactis ærogenes should very largely predominate.

If a sample is taken from the bottom of a lake it should contain mostly bacillus cloacæ.

A lake water should be condemned if it contains organisms of class I in I c.c. or less.

If fæcal organisms are present in the characteristic arrangement of fresh fæces.

If lactis ærogenes is scarce.

If the acid and gas line in glucose and lactose broth is the same, or close together.

If fæcal streptococci are found present.

In condemning a lake water, however, it should be remembered that even 48 hours will make an enormous difference to the purity of such a water, and the entire aspect of affairs may be changed within as short a period as one week.

RIVER WATERS.

A *good* river water may contain up to from 100 to 1,000 colonies (on agar at 37°C.).

Lactose fermenters may be present in 5 c.c.

Organisms of class I should not be present in 20 c.c.

Glucose fermenting organisms may be present in ·I c.c.

Fæcal organisms present should not contain a large number of different varieties in 10 colonies, and the prevalence of lactis ærogenes is a favourable sign.

River water should be condemned if it contains countless colonies (on agar at 37°C.).

If lactose fermenting fæcal organisms are present 10 to 100 per c.c.

If glucose fermenting organisms are present in same quantity.

If streptococci are present.

If the lactose fermenting organism shows class I in I to 5 c.c.

If the number of varieties is considerable in 10 colonies.

WELL AND SPRING WATER.

Good water should contain no lactose fermenting fæcal organism in 50 c.c.

No class I in 100 c.c.

Total colonies should be under 50 per c.c.

At present we are unable to give further standards than this.

Analyses characteristic of certain natural conditions in water.

(1) SAMPLE IV.—FROM A FOUNTAIN IN FRONT OF CHOULTRY NEAR RAILWAY STATION—1 MILE FROM SERVICE RESERVOIR—

(*a*) Total colonies on agar at 37°—110

(*b*) Bile salt lactose, medium—

	+	−
1 tube 20 cc. of water taken.	1	..
2 tubes 10 cc. of water taken.	2	..
4 tubes 5 cc. of water taken.	3	1
5 tubes 1 cc. of water taken.	1	4
5 tubes ·1 cc. of water taken.	..	5
5 tubes ·01 cc. of water taken.	..	5

(*c*) Fæcal bacilli in 5 cc. and upwards.

(*d*) Sporogenes milk test—in 20 cc.

Colony number.	Saccharose.	Dulcit.	Adonit.	Inulin.	Vosges and Prosk.	Motility.	Group.	Bacillus.
1	−	+	−	−	−	−	II	B. Coli communis.
2	−	−	−	−	−	−	I	B. Grunthal.
3	−	+	−	−	−	−	II	B. Coli communis.
4	+	−	−	−	−	−	IV	B. Coscoroba.
5	+	+	−	−	−	−	III	B. Neapolitanus.
6	+	−	−	−	−	−	IV	B. Coscoroba.
7	+	+	−	−	−	−	III	B. Neapolitanus.
8	+	+	−	−	−	−	III	Do.
9	+	+	−	−	−	−	III	Do.
10	+	+	−	−	−	−	III	Do.

(2) SAMPLE II.—A SHALLOW WELL ON THE BANK OF THE KALYANI RIVER WEST OF THE HEAD WORKS—AT A DISTANCE OF 320 FEET FROM THE 1ST UNFILTERED WATER—

(*a*) Total colonies on agar at 37°—3,150

(*b*) Bile salt lactose, medium—

	+	−
1 tube 20 cc. of water taken.	1	..
2 tubes 10 cc. of water taken.	1	1
4 tubes 5 cc. of water taken.	..	4
5 tubes 1 cc. of water taken.	..	5
5 tubes ·1 cc. of water taken.	..	5
5 tubes ·01 cc. of water taken	..	5

(*c*) Fæcal bacilli in 20 cc. and upwards.

(*d*) Sporogenes milk test—in 20 cc.

Colony number.	Saccharose.	Dulcit.	Adonit.	Inulin.	Vosges and Prosk.	Motility.	Group.	Bacillus.
1	−	−	−	−	−	−	I	B. Grunthal.
2	+	−	−	−	+	−	IV	B. Cloacæ.
3	+	−	−	−	+	+	IV	Do.
4	−	+	−	−	−	−	II	B. Coli communis.
5	−	−	−	−	−	+	I	B. Grunthal.
6	−	+	−	−	−	−	II	B. Coli communis.
7	−	+	−	−	−	−	II	Do.
8	−	+	−	−	−	+	II	Do.
9	+	−	−	−	+	+	IV	B. Cloacæ.
10	−	−	−	−	−	+	I	B. Grunthal.

(1) A polluted pipe water due to defect in pipes—a bad mixture.

(2) A bad well water—note fewness of fæcal organisms but presence of class I.

Analyses characteristic of certain natural conditions in water—contd.

(3) SAMPLE IV.—BOOSIKAL CHANNEL—

(*a*) Total colonies on agar at 37°—170

(*b*) Bile salt lactose, medium—

	+	−
1 tube 20 cc. of water taken.	1	. .
2 tubes 10 cc. of water taken.	2	. .
4 tubes 5 cc. of water taken.	4	. .
5 tubes 1 cc. of water taken.	5	. .
5 tubes ·1 cc. of water taken.	1	4
5 tubes ·01 cc. of water taken.	. .	5

(*c*) Fæcal bacilli in 1 cc. and upwards.

(*d*) Sporogenes milk test—in 20 cc.

Colony number.	Saccharose..	Dulcit.	Adonit.	Inulin.	Vosges and Prosk.	Motility.	Group.	Bacillus.
1	−	+	−	−	−	−	II	B. Coli communis.
2	−	−	−	−	−	−	I	B. Grunthal.
3	−	+	−	−	−	−	II	B. Coli communis.
4	+	+	−	−	−	+	III	B. Neapolitanus.
5	−	−	−	−	−	+	I	B. Grunthal.
6	+	−	−	−	−	+	IV	B. Coscoroba.
7	−	−	−	−	−	+	IV	Do.
8	−	−	−	−	−	+	I	B. Grunthal.
9	−	+	−	−	−	−	II	B. Coli communis, etc.
10	−	−	−	−	−	+	IV	B. Coscoroba.

(4) SAMPLE I.—STORAGE RESERVOIR—UNFILTERED WATER—

(*a*) Total colonies on agar at 37°—630.

(*b*) Bile salt lactose medium—

	+	−
1 tube 20 cc. of water taken.	1	. .
2 tubes 10 cc. of water taken.	2	. .
4 tubes 5 cc. of water taken.	4	. .
5 tubes 1 cc. of water taken.	5	. .
5 tubes ·1 cc. of water taken.	1	4
5 tubes ·01 cc. of water taken.	. .	5

(*c*) Fæcal bacilli in 1 cc. and upwards.

(*d*) Sporogenes milk test—in 20 cc.

Colony number.	Saccharose..	Dulcit.	Adonit.	Inulin.	Vosges and Prosk.	Motility.	Group.	Bacillus.
1	+	+	−	−	+	−	III	No. 73.
2	+	+	+	−	+	−	III	No. 67.
3	+	+	−	−	+	−	III	No. 73.
4	+	−	−	−	+	−	IV	B. Cloacæ.
5	+	+	−	−	+	−	III	No. 73
6	+	+	+	−	+	−	III	No. 67.
7	+	+	−	−	+	−	III	No. 73.
8	+	+	+	−	+	−	III	No. 67.
9	+	+	+	−	+	−	III	Do.
10	+	−	−	−	+	−	IV	B. Cloacæ.

(3) A bad mixture in a surface channel.

(4) Fairly good lake water—note, bacilli belong to more resistant end of class II and class III.

Analyses characteristic of certain natural conditions in water—contd.

			Colony number.	Saccharose.	Dulcit.	Adonit.	Inulin.	Vosges and Prosk.	Motility.	Group.	Bacillus.
(5) SAMPLE II.—FIRST TAP AFTER FILTRATION—											
(*a*) Total colonies on agar at 37°—170. (*b*) Bile salt lactose, medium—	+	–									
1 tube 20 c.c. of water taken.	1	..	1	+	+	–	–	+	–	III	No. 73.
2 tubes 10 c.c. of water taken.	2	..	2	–	+	–	–	–	–	II	B. Coli communis.
4 tubes 5 c.c. of water taken.	4	..	3	–	+	–	–	–	–	II	Do.
5 tubes 1 c.c. of water taken.	1	4	4	–	–	–	–	–	–	I	B. Grunthal.
5 tubes ·1 c.c. of water taken.	..	5	5	+	–	–	–	–	–	IV	B. Coscoroba.
5 tubes ·01 c.c. of water taken.	..	5	6	–	–	–	–	–	–	I B.	Grunthal.
			7	–	+	–	–	–	–	II	B. Coli communis.
(*c*) Fæcal bacilli in 5 c.c. and upwards.			8	+	+	–	–	+	+	III	No. 75.
(*d*) Sporogenes milk test—in 20 c.c.			9	+	+	–	–	+	–	III	No. 73.
			10	–	+	–	–	–	–	II	B. Coli communis, etc.
(6) SAMPLE III.—EASTERNMOST TAP NEAR BEACH—			1	+	+	+	–	+	–	III	No. 67.
(*a*) Total colonies on agar at 37°—30. (*b*) Bile salt lactose, medium—	+	–	2	–	+	–	–	–	–	II	B. Coli communis.
			3	+	+	+	–	+	–	III	No. 67.
1 tube 20 c.c. of water taken.	1	..	4	–	+	–	–	–	–	II	B. Coli communis.
2 tubes 10 c.c. of water taken.	2	..	5	–	+	–	–	–	–	II	Do.
4 tubes 5 c.c. of water taken.	4	..	6	–	+	–	–	–	–	II	Do.
5 tubes 1 c.c. of water taken.	5	..	7	..	+	..	..	..	..	..	B. Fæcalis alkaligenes (?).
5 tubes ·1 c.c. of water taken.	1	4	8	+	+	–	–	+	–	III	No. 73.
5 tubes ·01 c.c. of water taken.	..	5	9	–	+	–	–	–	–	II	B. Coli communis.
			10	–	+	–	–	–	–	II	Do.
(*c*) Fæcal bacilli in 1 c.c. and upwards.											
(*d*) Sporogenes milk test—in 20 c.c.											

(5) Same water polluted with mixture of sub-soil water through leaky filters.

(6) Typical bad mixture same series as 4 and 6—note, presence still of bacilli Nos. 67 and 73 occurring in the lake.

Analyses characteristic of certain natural conditions in water—contd.

(7) SAMPLE II.—DODABETTA LOWER RESERVOIR—

(*a*) Total colonies on agar at 37°—5,040.

(*b*) Bile salt lactose, medium—

	+	−
1 tube 20 c.c. of water taken.	1	..
2 tubes 10 c.c. of water taken.	2	..
4 tubes 5 c.c. of water taken.	4	..
5 tubes 1 c.c. of water taken.	5	..
5 tubes ·1 c.c. of water taken.	..	5
5 tubes ·01 c.c. of water taken.	..	5

(*c*) Fæcal bacilli in 1 c.c. and upwards.

(*d*) Sporogenes milk test—in 20 c.c.

Colony number	Saccharose.	Dulcit.	Adonit.	Inulin.	Vosges and Prosk.	Motility.	Group.	Bacillus.
1	+	−	+	−	+		IV	B. Lactis ærogenes or capsulatus.
2	+	−	+	−	+	−	IV	Do.
3	+	+	+	−	+	−	III	No. 67.
4	+	+	+	−	+	−	III	Do.
5	+	−	+	−	+	−	IV	B. Lactis ærogenes or capsulatus.
6	+	−	+	−	+	−	IV	Do.
7	+	+	−	−	−	−	III	B. Neapolitanus.
8	+	−	−	−	+	−	IV	B. Cloacæ.
9	+	−	−	−	+	−	IV	Do.
10	+	−	−	−	+	−	IV	Do.

(8) SAMPLE I.—CHANNEL AT WEST END OF RED HILLS LAKE—

(*a*) Total colonies on agar at 37°—78.

(*b*) Bile salt glucose, medium—

	+	−
1 tube 20 c.c. of water taken.	1	..
2 tubes 10 c.c. of water taken.	2	..
4 tubes 5 c.c. of water taken.	4	..
5 tubes 1 c.c. of water taken.	5	..
5 tubes ·1 c.c. of water taken.	3	2
5 tubes ·01 c.c. of water taken.	..	5

(*c*) Fæcal bacilli in ·1 c.c.

(*d*) Sporogenes milk test doubtful in 20 c.c.

Colony number	Saccharose.	Dulcit.	Adonit.	Inulin.	Vosges and Prosk.	Motility.	Group.	Bacillus.
1	+	−	−	−	+	+	IV	B. Cloacæ.
2	+	−	−	−	+	+	IV	Do.
3	+	−	−	−	+	+	IV	Do.
4	+	−	−	−	+	+	IV	Do.
5	+	−	−	−	+	−	IV	Do.
6	+	−	−	−	+	+	IV	Do.
7	+	−	−	−	+	+	IV	Do.
8	+	−	−	−	+	+	IV	Do.
9	+	−	−	−	+	+	IV	Do.
10	+	−	−	−	+	+	IV	Do
11	+	−	−	−	+	+	IV	Do.
12	+	−	−	−	+	+	IV	Do.
13	+	−	−	−	+	−	IV	Do.
14	+	−	−	−	+	−	IV	Do.
15	+	−	−	−	+	−	IV	Do.

(7) Fair lake water; note, mixture mostly cloacæ and lactis ærogenes, in classes II and III.

(8) A lake very low, otherwise not very objectionable, lactose fermenters too many numerically.

Analyses characteristic of certain natural conditions in water—contd.

(9) SAMPLE II.—DISTRIBUTARY SYSTEM—A TAP NEAR CATHEDRAL—

(*a*) Total colonies on agar at 37°—630.
(*b*) Bile salt glucose, medium—

	+	−
1 tube 20 c.c. of water taken.	1	. .
2 tubes 10 c.c. of water taken.	2	. .
4 tubes 5 c.c. of water taken.	4	. .
tubes 1 c.c. of water taken.	2	3
5 tubes ·1 c.c. of water taken.	. .	5
5 tubes ·01 c.c. of water taken.	. .	5

(*c*) Fæcal bacilli in 1 c.c. and upwards.
(*d*) Sporogenes milk test— in 20 c.c.

Colony number.	Saccharose.	Dulcit.	Adonit.	Inulin.	Vosges and Prosk.	Motility.	Group.	Bacillus.
1	−	−	−	−	−	−	I	B. Grunthal.
2	−	−	−	−	−	−	I	Do.
3	−	−	−	−	−	−	I	Do.
4	+	−	−	−	+	−	IV	B. Cloacæ.
5	−	−	−	−	−	−	I	B. Grunthal.
6	−	−	−	−	−	−	I	Do.
7	+	−	−	−	+	−	IV	B. Cloacæ.
8	−	−	−	−	−	−	I	B. Grunthal.
9	−	−	−	−	−	−	I	Do.
10	+	−	−	−	+	−	IV	B. Cloacæ.
11	−	+	−	−	−	+	II	B. Coli, etc.
12	−	−	−	−	−	−	I	B. Grunthal.
13	−	+	−	−	−	+	II	B. Coli, etc.
14	−	+	−	−	−	+	II	Do.
15	+	−	−	−	−	+	IV	B. Coscoroba.
16	+	−	−	−	−	+	IV	B. Cloacæ.

(10) SAMPLE VI.—WATER OF THE RED HILLS LAKE—

(*a*) Total colonies on agar at 37°—160.
(*b*) Bile salt lactose, medium—

	+	−
1 tube 20 c.c. of water taken.	1	. .
2 tubes 10 c.c. of water taken.	2	. .
tubes 5 c.c. of water taken.	4	. .
5 tubes 1 c.c. of water taken.	. .	5
5 tubes ·1 c.c. of water taken.	. .	5
5 tubes ·01 c.c. of water taken.	. .	5

(*c*) Fæcal bacilli in 5 c.c. and upwards.
(*d*) Sporogenes milk test + in 20 c.c.

Colony number.	Saccharose.	Dulcit.	Adonit.	Inulin.	Vosges and Prosk.	Motility.	Group.	Bacillus.
1	+	−	+	−	+	−	IV	B. Lactis ærogenes.
2	+	−	+	−	+	−	IV	Do.
3	+	−	+	−	+	−	IV	Do.
4	+	−	+	−	+	−	IV	Do.
5	+	−	+	−	+	−	IV	Do.
6	+	−	+	-	+	−	IV	Do.
7	+	−	+	−	+	−	IV	Do.
8	+	−	+	−	+	−	IV	Do.
9	+	−	+	−	+	−	IV	Do.
10	+	−	−	−	−	+	IV	B. Coscoroba.

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(9) Same water as (8) with pollution added since water left lake.
(10) A typical fair quality lake. Water—note, moderate number of local fæcal organisms and preponderance of lactis ærogenes.

Analyses characteristic of certain natural conditions in water—contd.

			Colony number.	Saccharose.	Dulcit.	Adonit.	Inulin.	Vosges and Prosk.	Motility.	Group.	Bacillus.
(11) Sample I.—Samalkota Canal—											
(*a*) Total colonies on agar at 37°—1,890. (*b*) Bile salt lactose, medium—	+	−									
1 tube 20 c.c. of water taken.	1	..									
2 tubes 10 c.c. of water taken.	2	..	1	—	—	—	—	—	—	I	B. Grunthal.
4 tubes 5 c.c. of water taken.	3	1	2	—	—	—	—	—	—	I	Do.
5 tubes 1 c.c. of water taken.	3	2	3	—	—	—	—	—	—	I	Do.
5 tubes ·1 c.c. of water taken.	..	5	4	—	—	—	—	—	+	I	Do.
5 tubes ·01 c.c. of water taken.	..	5	5	—	—	—	—	—	—	I	Do.
			6	—	—	—	—	—	—	I	Do.
(*c*) Fæcal bacilli in 1 c.c. and upwards.			7	—	—	—	—	—	—	I	Do.
(*d*) Sporogenes milk test—in 20 c.c.			8	—	—	—	—	—	—	I	Do.
			9	—	—	—	—	—	—	I	Do.
			10	—	—	—	—	—	—	I	Do.
(12) Sample II.—Storage Tank—Unfiltered Water—											
(*a*) Total colonies on agar at 37°—170.	+	−	1	—	—	—	—	—	—	I	B. Grunthal.
(*b*) Bile salt lactose, medium—			2	—	—	—	—	—	—	I	Do.
1 tube 20 c.c. of water taken.	1	..	3	—	—	—	—	—	—	I	Do.
2 tubes 10 c.c. of water taken.	2	..	4	—	—	—	—	—	+	I	Do.
4 tubes 5 c.c. of water taken.	..	4	5	—	—	—	—	—	—	I	Do.
5 tubes 1 c.c. of water taken.	..	5	6	—	—	—	—	—	—	I	Do.
5 tubes ·1 c.c. of water taken.	..	5	7	—	—	—	—	—	—	I	Do.
5 tubes ·01 c.c. of water taken.	..	5	8	—	—	—	—	—	—	I	Do.
			9	—	—	—	—	—	—	I	Do.
(*c*) Fæcal bacilli in 10 c.c. and upwards.			10	—	—	—	—	—	—	I	Do.
(*d*) Sporogenes milk test—in 20 c.c.											

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(11) A typical well sunned water in river (irrigation canal)—note, improvement between (11) and (12).

(12) A typical well sunned water in settling tank—note, improvement between (11) and (12).

Analyses characteristic of certain natural conditions in water—contd.

(13) SAMPLE I.—FROM THE RESERVOIR AT—

(*a*) Total colonies on agar at 37°—69 unfiltered.	—	—
(*b*) Bile salt glucose, medium—	—	—
1 tube 20 c.c. of water taken.	1	..
2 tubes 10 c.c. of water taken.	2	..
4 tubes 5 c.c. of water taken.	4	..
5 tubes 1 c.c of water taken.	5	..
5 tubes ·1 c.c. of water taken.	2	3
5 tubes ·01 c.c. of water taken.	2	3

(*c*) Fæcal bacilli in 1 c.c. and upwards

(*d*) Sporogenes milk test doubtful in 20 c.c.

Colony number.	Saccharose.	Dulcit.	Adonit.	Inulin.	Vosges and Prosk.	Motility.	Group.	Bacillus.
1	—	—	—	—	+	—	IV	B. Cloacæ.
2	+	—	—	—	+	—	IV	Do.
3	+	—	—	—	+	—	IV	Do.
4	+	—	—	—	+	—	IV	Do.
5	+	—	—	—	+	—	IV	Do.
6	+	—	—	—	—	—	IV	Do.
7	+	—	—	—	+	—	IV	Do.
8	+	—	—	—	+	—	IV	Do.
9	+	—	—	—	+	+	IV	Do.
10	+	—	—	—	+	+	IV	Do.
11	+	—	—	—	+	—	IV	Do.
12	+	—	—	—	+	—	IV	Do.

(14) SAMPLE I.—FROM THE RIVER-BED—

(*a*) Total colonies on agar at 37°—Countless (in 1 c.c.).	—	—
(*b*) Bile salt lactose, medium—	—	—
1 tube 20 c.c. of water taken	1	..
2 tubes 10 c.c. of water taken.	2	..
4 tubes 5 c.c. of water taken	4	..
5 tubes 1 c.c. of water taken	5	..
5 tubes ·1 c.c. of water taken	5	..
5 tubes ·01 c.c. of water taken	1	4

(*c*) Fæcal bacilli in ·1 c.c. and upwards

(*d*) Sporogenes milk test—in 20 c.c.

Colony number.	Saccharose.	Dulcit.	Adonit.	Inulin.	Vosges and Prosk.	Motility.	Group.	Bacillus.
1	+	+	—	—	—	—	IV	B. Cloacæ.
2	+	+	—	—	—	—	IV	Do.
3	+	—	—	—	—	—	IV	B. Coscoroba.
4	+	—	—	—	—	—	I	B. Grunthal.
5	+	+	+	+	—	—	III	No. 69.
6	+	—	—	—	—	—	IV	B. Coscoroba.
7	+	+	+	+	+	—	III	B. Oxytocus Perniciosus.
8	—	—	—	—	—	—	I	B. Grunthal.
9	—	—	—	—	—	—	I	Do.
10	+	—	—	—	—	—	IV	B. Coscoroba.

(13) Lake—Very low indeed; no rain for 8 months. Organisms rather too numerous.

(14) Typical bad mixtures in river water; note, prevalence of class 1 and mixtures of organisms.

Analyses characteristic of certain natural conditions in water—concld.

(15) SAMPLE I.—FROM THE RUNNING WATER OF THE VAIGAI RIVER (UNFILTERED WATER)—

(*a*) Total colonies on agar at 37°—Countless.	—	—
(*b*) Bile salt lactose, medium—	—	—
1 tube 20 c.c. of water taken.	1	..
2 tubes 10 c.c. of water taken.	2	..
4 tubes 5 c.c. of water taken.	4	..
5 tubes 1 c.c. of water taken.	5	..
5 tubes ·1 c.c. of water taken.	5	..
5 tubes ·01 c.c. of water taken.	5	..

(*c*) Fæcal bacilli in ·01 c.c. and upwards.

(*d*) Sporogenes milk test—in 20 c.c.

Colony number.	Saccharose.	Dulcit.	Adonit.	Inulin.	Vosges and Prosk.	Motility.	Group.	Bacillus.
1	−	+	−	−	−	−	II	B. Coli communis.
2	−	+	−	−	−	−	II	Do.
3	−	+	−	−	−	−	II	Do.
4	−	+	−	−	−	−	II	Do.
5	−	+	−	−	−	−	II	Do.
6	−	+	−	−	−	−	II	Do.
7	−	−	−	−	−	−	I	B. Grunthal.
8	−	−	−	−	−	−	I	Do.
9	+	+	−	−	−	−	III	B. Neapolitanus.
10	−	+	−	−	−	−	II	B. Coli communis, etc.

(16) SAMPLE I.—WATER FROM ABOVE THE INFILTRATION GALLERY—UNFILTERED WATER—

(*a*) Bile salt lactose, medium—		
1 tube of 20 c.c. of water taken ..	— 1	— ..
2 tubes of 10 c.c. of water taken ..	2	..
4 tubes of 5 c.c. of water taken ..	4	..
5 tubes of 1 c.c. of water taken ..	5	..
5 tubes of ·1 c.c. of water taken ..	1	4
5 tubes of ·01 c.c. of water taken ..	..	5

(*b*) Fæcal bacilli in 1 c.c. and upwards.

(*c*) Sporogenes milk test—in 20 c.c.

Colony number.	Saccharose.	Dulcit.	Adonit.	Inulin.	Vosges and Prosk.	Motility.	Group.	Bacillus.
1	−	−	−	−	−	−	I	B. Grunthal.
2	+	−	−	−	−	−	IV	B. Coscoroba.
3	+	+	+	−	−	−	III	B. Pneumoniæ or Rhinoscleromatis.
4	+	−	+	−	+	−	IV	B. Lactis ærogenes or capsulatus.
5	−	+	−	−	−	−	II	B. Coli communis.
6	−	+	−	−	−	−	II	Do.
7	−	−	−	−	−	−	I	B. Grunthal.
8	−	+	−	−	−	−	II	B. Coli communis.
9	+	−	−	−	−	−	IV	B. Coscoroba.
10	+	+	+	−	−	−	III	No. 73.

(15) and (16) Typical bad mixtures in river waters; note prevalence of class I and mixtures of organisms.

CHAPTER XII.

THE "COLI" METHOD COMPARED WITH THE SEPARATE SPECIES METHOD.

From what has gone before it will be apparent that, rightly or wrongly, we appear to make use of very much more information in the judging of a sample of water than is customary amongst analysts at home. It would be well therefore to make a few remarks on what we may call the "coli" method of judging water.

The term "coli communis" is, as MacConkey says, not a happy one because no two bacteriologists use the term in the same sense. Four interpretations of this term are given here.

(1) The report of the English Committee on the standardization of methods in the examination of water defines the characters of coli communis as follows :—A small motile non-sporing bacillus growing at 37° C. as well as at room temperature. The motility is well observed in young cultures in a fluid glucose medium. It is decolourized by Gram's method of staining. It never liquefies gelatine, and the cultures should be kept for at least 10 days in order to exclude a liquefying bacillus. It forms smooth thin surface growths and colonies on gelatine—not corrugated—growing well to the bottom of the stab (facultative anærobe). It produces permanent acidity in milk which it curdles within 7 days at 37° C. It ferments glucose and lactose with the production of both acid and gas. The typical coli bacillus must conform to the above description and tests. It generally also forms indol (best obtained in peptone water cultures), gives a big yellowish brown growth on potato (greatly depends on the character of the potato), sometimes ferments saccharose (about 50 per cent.), changes neutral red, reduces nitrates and half the gas produced by it from glucose is absorbable by caustic potash.

(2) The American Committee on standard methods states, that the test for the presence of this bacillus in water

cannot be considered complete unless the organism is isolated in pure culture, and is found to show the following characters :—Typical morphology, *i.e.*, a non-sporing bacillus relatively small and often quite thick ; motility, when a young growth on gelatine culture is examined ; non-liquefaction of gelatine ; fermentation of glucose broth, with the formation of about 50 per cent. of gas of which about $\frac{1}{3}$ (CO_2) is absorbed by 2 per cent. solution sodium hydrate ; coagulation of milk with the production of acid in 48 hours or more at 37° C., either spontaneously or on boiling ; production of indol in peptone solution ; reduction of nitrates.

(3) Houston describes "Coli communis" as follows :—The organism is motile ; gives gas in gelatine shake cultures ; acid and gas in both glucose and lactose peptone media ; positive result with neutral red broth ; indol formed ; acid and clot in litmus milk ; gas acid and clot in lactose peptone milk cultures ; reduction of nitrates ; bright cherry red colour in Proskauer and Capaldi medium No. I ; bleached appearance and no definite acidity in Proskauer and Capaldi medium No. II ; percentage acidity in litmus whey culture = 28 to 46 c.c. N. 10, Na_2CO_3.

(4) Savage gives the following tests as both essential and sufficient to identify true bacillus coli communis for purposes of water analysis. A short bacillus with rounded ends ; no spores ; motile ; decolourized by Gram's method ; characteristic growth on gelatine slope ; acid production and coagulation in litmus milk ; production of indol ; production of considerable quantity of acid in litmus whey ; fermentation with production of both gas and acid in glucose and lactose media ; no fermentation in starch or saccharose ; a positive neutral red reaction.

From the work previously described it is certain that (i) Houston's true coli is not a single organism at all but a group of some ten or a dozen bacilli, and (ii) there is an enormous number of bacilli—such as the saccharose fermenters—in fæces that must be called "atypical coli," because they only partially fulfil the above conditions.

It would appear that this arrangement of classifying fæcal bacilli into "true coli" and "atypical coli" is open to two objections : (1) That it is inadvisable to apply the term " coli communis " to a group of organisms, or even so-called varieties, and (2) The tests laid down as essential according to the definitions quoted, do not go far enough ; the list contains too many that are of doubtful significance and utility, and too few that are admittedly of greater importance in the identification of a bacillus. To discuss this last objection first : Houston, Savage and all experienced bacteriologists admit, that the tests given above are not of equal value in the identification of what they term " coli." Thus to quote from Savage, " Different characters have not an equal value. Some, such as the fermentation of sugars, liquefaction of gelatine, and acid production in milk are of so permanent a type that for an isolated organism to show divergence from a typical bacillus coli in any one of these particulars would be to throw grave doubt on its being excretal at all, while others, such as the gelatine surface colonies, and the exhibition of motility, are so subject to variation that comparatively little significance can be attached to their, perhaps temporary, absence or modification." And again in another place he says : " Organisms that differ from the typical excretal type (bacillus coli communis) only in the fact that they show no motility and that their gelatine colonies are atypical may be regarded as not having their significance diminished on these grounds. Similarly the loss of indol-production power would not reduce their significance very markedly. On the other hand, the absence of glucose or lactose fermentation would at once, in the writer's opinion, exclude the organism isolated from being bacillus coli at all."

The obvious inference from opinions is, why not do away with some of these doubtful tests and substitute for them the more important fermentation reactions in sugars such as dulcite, adonite and inulin ? These are entirely on a parallel with the glucose and lactose fermentation referred to as being " of a permanent character." This is the position taken up by MacConkey, in his paper on the Bacteriology of Milk (Journal of Hygiene, Vol. IV, No. 3, July 1906), and, following him by Bergey and Deehan, Orr and ourselves.

But the real objection to calling a group of bacilli, made up of definite and distinct species, by the single term "coli" becomes more apparent when an attempt is made to interpret the results obtained by these methods. It is accepted that as long as a bacillus fulfils all the tests in the previous quotations it is looked upon as fæcal; but the difficulty arises when bacilli are isolated which either do not fulfil all the above conditions, or possess additional characteristics. Thus a fair number of the so-called "coli group" are non-motile; some do not produce indol; others ferment saccharose. The difficulty is to estimate what is the significance of these aberrant or atypical varieties, and it is this point which demonstrates the weakness of the above system. Thus authorities interpret these facts differently: Houston says that the saccharose fermenting organisms are not to be looked upon as so "significant of undesirable pollution" as the non-saccharose fermenters. Savage, on the other hand, says that they are equally significant of fæcal contamination. Here we have two contradictory opinions on a most vital point. In discussing this difficulty in estimating the value of these aberrant types, Savage says: "undoubtedly this question of variation and loss of attributes complicates the subject and detracts from the value of this indicator" (*i.e.*, "coli" as an indicator of fæcal contamination). Again, "There is the great difficulty which attaches to the precise significance given to the bacilli which deviate from the characters ascribed to the coli communis." Surely, therefore, it would be better to separate these organisms, by the use of well-defined fermentation reactions into different species, and to study their position in nature. By the use of MacConkey's method we can not only easily recognize the individual varieties of fæcal bacilli in a water, but we have shown in the early part of this book that some of these have very important natural characteristics which have nothing whatever to do with their laboratory reactions, and which are of great value to the water analyst. This knowledge could not have been acquired without separating species. As long as the method of calling all organisms that fulfil a few tests, "coli," and those that do not fulfil them, or only fulfil them in part, " atypical coli," the possibilities of progress are very much reduced. Although it may be true that this method has, in skilful hands in Europe, given satisfactory results, it is obvious that unless each species of bacterium is isolated and studied separately, both as regards its position in nature and as regards its prevailing characteristics, we shall remain in our present position, talking of " coli " and " atypical coli " permanently.

In order to demonstrate the unsuitability of the definition of " coli," and of the standards made use of in common practice in Europe and America, when applied to the Madras Presidency, it will be well to observe the result of applying them to the samples examined in our laboratory. Houston's " true coli " ferments lactose, ferments glucose, does not ferment saccharose, gives indol reaction, and the other tests already described. Organisms that fulfil these tests are indicative of fæcal contamination and are more objectionable than organisms that ferment saccharose. By making use of MacConkey's method it is possible to split up this " true coli " of Houston into about 10 different bacilli (*vide* Table XII(*a*)), and if we add his " non-typical coli " (that is to say, those that do not give the indol reaction), more are added. Now it must be remembered that *all these organisms are looked upon as indicating the worst form of contamination.* Apart from the objection of classing 10 different organisms under the term " true coli," it has never been demonstrated that the members of this group are *equally* objectionable, or in other words, *equally* represent recent and serious contamination. We have already shown that this is not the case.

Savage's definition of " true coli " is virtually the same as Houston's, but as regards the interpretation, he makes this statement " all workers class both groups (saccharose and non-saccharose fermenters) as of equal value as excretal indicators." Consequently all lactose fermenters except such as liquefy gelatine, do not give the indol reaction, and are non-motile (*vide* his previous remark on motility, indol, etc.) may be said to be of equal value as indicators of fæcal contamination. Therefore, there are, according to the table, 10 or 12 organisms that fulfil all these necessary conditions; if motility and indol are left out, there are more than 20 bacilli, to be regarded as equally objectionable in a water sample.

To take Houston's definition of true coli first: of the 239 samples, which have been worked through in our laboratory, derived from 18 different water-supplies, in 3 separate quarters of 1909, only 84 would show no "coli." If Savage's wider statement be accepted, it means that we have practically not a single supply in the Madras Presidency that does not show objectionable fæcal contamination. Is it reasonable to urge that we in India should have condemned all these different water-supplies?

There is another point that demands notice. Take the organism bacillus cloacæ as an example; it will be observed that this organism does not come within Houston's definition of "true coli," because it ferments saccharose; nor within Savage's group that indicates fæcal contamination, because it liquefies gelatine, yet it is undoubtedly fæcal in origin. Why should the mere fact that this organism liquefies gelatine mean that it does not indicate fæcal contamination to an equal extent as any non-liquefier? Surely, considering it originally came from the fæces of man or some other animal, the coincidence that it liquefies gelatine cannot put it beyond the range of indicators of fæcal contamination. As a matter of fact, B. cloacæ, when found by itself in samples, does not indicate recent and dangerous pollution; it is, as we have seen, a resistant organism, with a fondness for the bottom of lakes, and river sand; but the liquefaction of gelatine has nothing to do with these facts.

The weak point of the above method lies in the statement that a large number of what we believe to be entirely different species of bacilli *equally represent dangerous fæcal contamination*. If this be so, it should be demonstrable that these different species are equally resistant to such forces of nature as the action of sunlight and the action of storage. Otherwise if it can be proved that one organism is particularly resistant, and another particularly susceptible to these natural forces, it is obvious that the resistant organisms cannot be considered, on all occasions, of equal value as an index of recent and dangerous pollution, as the susceptible one. Examining the ten organisms, which fall under Houston's definition of "coli," and which represent his worst type of pollution, we have shown that the greatest possible variations exist in these ten organisms in their power of resistance to the action of natural forces. To take two extreme examples, one member of the Grunthal-Vesiculosus group is particularly resistant to the action of sunlight. It is found in waters that have been exposed to the tropical sun for weeks; it is nearly always one of the last bacilli to disappear from a well-sunned and stored water, and also from a mixture of cowdung and water exposed to the sun. On the other hand, true B. coli communis of Escherich (the organism that ferments dulcit and does not ferment saccharose, adonit, inulin, etc.) will disappear with great rapidity from a water exposed to the action of tropical sun. Is it reasonable to include both these bacilli under the same term of "true coli," and to state that both equally represent objectionable pollution?

It is possible to elaborate this still further. Take the example of Oxytocus perniciosus: we have pointed out that this organism is susceptible to natural conditions, such as sunlight, etc., for although it is present in cowdung, and in human fæces, it has only been found in water-supplies after heavy floods, and it disappears with extraordinary rapidity. Thus, it is probable that this organism represents a very recent, and therefore a more objectionable contamination than any other organism met with up to now. Yet it will be observed that this particular bacillus does not fall within Houston's definition of "true coli," nor within the category of organisms which according to Savage are "equally of excretal origin." In order to demonstrate what is exactly meant by this last paragraph, reference should be made to the analysis of the water of the District Jail of Tanjore, given here.

The water contains fæcal bacilli in ·01 c.c. It contains sporogenes in 20 c.c., and the total colonies in 1 c.c. at 37°C were 2,520. The only bacillus that would fulfil the definition of "coli" according to Houston is B. Grunthal, an organism

Sample I.—Reservoir of the well.

(*a*) Total colonies on agar at 37°C —2,520.

(*b*) Bile salt lactose, medium.

	+	−
1 tube 20 c.c. of water taken ..	1	..
2 tubes 10 c.c. ,, ..	2	..
4 ,, 5 c.c. ,, ..	4	..
5 ,, 1 c.c. ,, ..	5	..
5 ,, ·1 c.c. ,, ..	5	..
5 ,, ·01 c.c. ,, ..	5	..

(*c*) Fæcal bacilli in ·01 c.c. and upwards.

(*d*) Sporogenes milk test — in 20 c.c.

Colony No.	Saccharose.	Dulcit.	Adonit.	Inulin.	Vosges and Frosk.	Motility.	Group.	Bacillus.	Group and Remarks.
1	+	−	−	−	+	−	IV	B. Cloacæ.	Group I. 2.
2	+	+	−	+	+	−	III		,, II, *nil*.
3	−	−	−	−	−	−	I	B. Grunthal.	,, III, 5.
4	+	+	+	+	+	−	III	Perniciosus.	,, IV. 5.
5	+	+	−	−	+	−	III	B. Oxytocus.	
6	+	+	−	+	+	−	III		
7	+	−	−	−	+	−	IV	B. Cloacæ.	
8	−	−	−	−	−	−	I	B. Grunthal.	
9	+	+	−	+	−	−	III		
10	+	−	−	−	−	−	IV	B. Coscoroba.	

which we know to be particularly resistant, and which, when found by itself, is of little significance as an indicator of recent pollution. But the organisms Nos. 2, 6 and 9 and the Oxytocus perniciosus fall in with neither Savage's nor Houston's definition of "coli," yet there is good reason to believe that these bacilli are much more specifically an indication of recent pollution than true B. coli communis of Escherich itself.

A second example illustrating this important point will be found by referring to the analysis of Cocanada water, given below. The water at Cocanada is originally taken from an irrigation canal; it is run into a storage tank where it remains for anything from seven days to a month; it is then filtered and pumped into the town. The canal sample contains fæcal bacilli in 1 c.c.; but having been exposed to the sun for a considerable time, as the water flows slowly for many miles down a shallow irrigation canal, it has been so thoroughly well sunned and settled, that the only organism present is B. Grunthal. After the water has remained in the storage tank, this organism has further decreased in number, till it is only present in 10 c.c. of the water. *Now these two samples would be condemned as containing coli.* The water then passes through filter beds. As a result of this would-be filtration, the fæcal bacilli have run up from 1 in 10 c.c. to 1 in ·3 of a c.c. *None of these samples after filtration contain any organism which can be called "true coli."* It has been shown by inspection that the reason for this increase is due to the fact that a polluted subsoil water finds its way through the masonry floor of the filter beds. Therefore there can be no doubt that this admixture is distinctly undesirable, though the bacilli added happen to be mainly B. cloacæ—an organism which neither Houston nor Savage include within their definition "true coli," and which (because it liquefies gelatine) would be regarded as of no serious importance as an indicator of fæcal contamination. We do not suppose that either Dr. Houston or Savage would have overlooked the great increase in the number of organisms that gave acid and gas in lactose broth without some inquiry, but this case demonstrates the fact that the generalization concerning the interpretation of

COCANADA SEPT. 18, 1911.

Bacteriological Analysis.

SAMPLE I.—SAMALKOTA CANAL.

(*a*) Total colonies on agar at 37°—1,890.
(*b*) Bile salt lactose, medium.

	+	—
1 tube 20 c.c. of water taken ..	1	..
2 tubes 10 c.c. ,, ..	2	..
4 ,, 5 c.c. ,, ..	3	1
5 ,, 1 c.c. ,, ..	3	2
5 ,, ·1 c.c. ,, ..	..	5
5 ,, ·01 c.c. ,, ..	..	5

(*c*) Fæcal bacilli in 1 c.c. and upwards.
(*d*) Sporogenes milk test — in 20 c.c.

Colony No.	Saccharose.	Dulcit.	Adonit.	Inulin.	Vosges and Prosk.	Mobility.	Group.	Bacillus.	Group and Remarks.
1	—	—	—	—	—	—	I	B. Grunthal.	Group I 10.
2	—	—	—	—	—	—	I	Do.	,, II *nil.*
3	—	—	—	—	—	—	I	Do.	,, III *nil.*
4	—	—	—	—	—	—	I	Do.	,, IV *nil.*
5	—	—	—	—	—	—	I	Do.	
6	—	—	—	—	—	—	I	Do.	
7	—	—	—	—	—	—	I	Do.	
8	—	—	—	—	—	—	I	Do.	
9	—	—	—	—	—	—	I	Do.	
10	—	—	—	—	—	—	I	Do.	

SAMPLE II.—STORAGE TANK—UNFILTERED WATER.

(*a*) Total colonies on agar at 37°—170.

(*b*) Bile salt lactose, medium.

	+	—
1 tube 20 c.c. of water taken ..	1	..
2 tubes 10 c.c. ,, ..	2	..
4 ,, 5 c.c. ,, ..	..	4
5 ,, 1 c.c. ,, ..	..	5
5 ,, ·1 c.c. ,, ..	..	5
5 ,, ·01 c.c. ,, ..	..	5

(*c*) Fæcal bacilli in 10 c.c. and upwards.
(*d*) Sporogenes milk test — in 20 c.c.

Colony No.	Saccharose.	Dulcit.	Adonit.	Inulin.	Vosges and Prosk.	Mobility.	Group.	Bacillus.	Group and Remarks.
1	—	—	—	—	—	—	I	B. Grunthal.	Group I 10.
2	—	—	—	—	—	—	I	Do.	,, II *nil.*
3	—	—	—	—	—	—	I	Do.	,, III *nil.*
4	—	—	—	—	—	+	I	Do.	,, IV *nil.*
5	—	—	—	—	—	—	I	Do.	
6	—	—	—	—	—	—	I	Do.	
7	—	—	—	—	—	—	I	Do.	
8	—	—	—	—	—	—	I	Do.	
9	—	—	—	—	—	—	I	Do.	
10	—	—	—	—	—	—	I	Do.	

Bacteriological Analysis—Contd.

SAMPLE III.—COLLECTING WELL AFTER FILTER BEDS—FILTERED WATER.

(*a*) Total colonies on agar at 37°—300.

(*b*) Bile salt lactose, medium.

	+	—
1 tube 20 c.c. of water taken ..	1	..
2 tubes 10 c.c. ,, ..	2	..
4 ,, 5 c.c. ,, ..	4	..
5 ,, 1 c.c. ,, ..	3	2
5 ,, ·1 c.c. ,, ..	2	3
5 ,, ·01 c.c. ,, ..	..	5

(*c*) Fæcal bacilli in ·3 cc. and upwards.
(*d*) Sporogenes milk test — in 20 cc.

Colony No.	Saccherose.	Dulcit.	Adonit.	Inulin.	Vosges and Prosk.	Mobility.	Group.	Bacillus.	Group and Remarks.
1	+	—	—	—	+	+	IV	B. Cloacæ.	Group I *nil.*
2	+	—	—	—	+	+	IV	Do.	,, II *nil.*
3	+	—	—	—	+	+	IV	Do.	,, III *nil.*
4	+	—	—	—	+	+	IV	Do.	,, IV 10
5	+	—	—	—	+	+	IV	Do.	
6	+	—	—	—	+	+	IV	Do.	
7	+	—	—	—	+	+	IV	Do.	
8	+	—	—	—	+	+	IV	Do.	
9	+	—	—	—	+	+	IV	Do.	
10	+	—	—	—	+	+	IV	Do.	

SAMPLE V.—FOUNTAIN IN FRONT OF VETERINARY HOSPITAL.

(*a*) Total colonies on agar at 37°—3,150.

(*b*) Bile salt lactose, medium.

	+	—
1 tube 20 c.c. of water taken ..	1	..
2 tubes 10 c.c. ,, ..	2	..
4 ,, 5 c.c. ,, ..	4	..
5 ,, 1 c.c. ,, ..	3	2
5 ,, ·1 c.c. ,, ..	..	5
5 ,, ·01 c.c. ,, ..	..	5

(*c*) Fæcal bacilli in 1 cc. and upwards.
(*d*) Sporogenes milk test — in 20 cc.

Colony No.	Saccherose.	Dulcit.	Adonit.	Inulin.	Vosges and Prosk.	Mobility.	Group.	Bacillus.	Group and Remarks.
1	+	—	—	—	+	—	IV	B. Cloacæ.	Group I *nil.*
2	+	—	—	—	+	—	IV	Do.	,, II *nil.*
3	+	—	—	—	+	—	IV	Do.	,, III *nil.*
4	+	—	—	—	+	—	IV	Do.	,, IV 10.
5	+	—	—	—	+	—	IV	Do.	
6	+	—	—	—	+	—	IV	Do.	
7	+	—	—	—	+	—	IV	Do.	
8	+	—	—	—	+	—	IV	Do.	
9	+	—	—	—	+	—	IV	Do.	
10	+	—	—	—	+	—	IV	Do.	

so-called "true coli" not infrequently misses the mark. Many other instances could be quoted, but these will suffice.

Therefore we think it will be conceded that it is not satisfactory to arrange different organisms into groups under one name, and that it is still more dangerous to make statements relative to the significance of the group as a whole, because these statements frequently prove incorrect for the individual members.

The division of organisms into groups according to fermentation reactions is a necessary part of laboratory routine; but it should not be forgotten, that this method of classification tells us nothing of the many characteristics of the organisms. Thus taking the group of lactose fermenters as a whole, they may be split up into sub-groups innumerable, but it is none the less necessary to separate species and to study their natural characteristics. The danger in generalizing, with the fermentation method as a basis of classification, applies to *all* groups of organisms, including those suggested by MacConkey.

It may reasonably be asked how does it come about that English methods have given such satisfactory results as to lead to their general adoption. The answer is that in waters in England *practically any trace of pollution is dangerous.* As we have pointed out in the first chapter, there is not time for any very elaborate self-purification to go on in most natural sources, and a more detailed examination of the process of storage on the lines suggested by us would probably show that the results obtained in England resembled those in this country. Houston's figures quoted in Chapters VI—X, undoubtedly confirm this statement. At any rate it may be stated that the problem is so entirely different in the two countries that it would be ridiculous to suppose that English standards are suited to the tropical conditions. The table given below gives the results in three quarters of what would occur if English standards were applied to the waters in this country.

	Quarter ending 30th September 1908.	Quarter ending 31st December 1908.	Quarter ending 31st March 1909.
(1) Number of samples examined ..	76	85	78
(2) Number of samples that contain “ Coli ” according to Houston ..	63	57	45
(3) Number of samples that contain true bacillus coli communis of Escherich ..	13	13	11
(4) Number of samples in which the only organism that falls under the definition of Houston's “ Coli ” is bacillus Grunthal	25	22	22
(5) Number of samples containing mixtures of Grunthal, acidi lactici, and others of Houston's “ Coli ” group	25	22	12
(6) Number containing no “ Coli ” of Houston	13	28	33

It will be observed that an enormous percentage of the supplies would be condemned entirely unnecessarily.

The recognition of truly recent pollution is, we maintain, easy by using certain tests ; but it is perfectly obvious that the application of the “ true coli ” and “ atypical coli ” method would tell us nothing in these samples.

The terms “ true and atypical coli ” are, we maintain, purely a laboratory classification, they are not in the slightest degree based on a study of the natural characteristics of fæcal organisms, and in estimating the age of a pollution they are of no value ; indeed we consider that just as much information as to the quality of the water can be obtained by roughly estimating the number of lactose fermenting organisms that are present in any water, without saying whether they belong to the “ true or atypical ” class of coliform bacilli.

Finally, as long ago as 1905, MacConkey recommended that all fæcal bacilli in a water sample should be separated and studied, and that by doing so, we should probably greatly increase our knowledge of the true significance of the presence of these micro-organisms.

We consider that we have amply proved the truth of these contentions, and the utility of his method.

Laying aside for the time being all the more debatable part of our work—such as, the difference in powers of resistance of various bacilli—as not yet proven, who ever heard it even suggested, that by the "coli" method, it would be possible for an analyst to say from his results if a water was taken from a lake nearly empty, or from a well after long drought? Who ever reported that such-and-such a mixture of fæcal organisms was highly suggestive of fresh fæces or that such another mixture suggested that some alteration from natural conditions had occurred? Was it ever seriously put forward that bacillus cloacæ, lactis ærogenes and oxytocus perniciosus might be as valuable in indicating a certain definite stage in pollution as "true coli" itself? Would it have been possible, working with the "true coli" method, to discover the many interesting problems connected with animal and human fæces mentioned in this work?

An honest answer to these queries should convince any unbiassed mind, that in adhering rigidly to the presence of "coli" as an indication of pollution, and being content therewith, we are deliberately shutting our eyes to a very fruitful field of knowledge which is of great value in the study of fæcal pollution generally.

TABLE XII (*a*).

After MacConkey (1909).

No.	——	Lactose.	Saccharose.	Dulcit.	Adonit.	Inulin.	P. & V. reaction.	Indol.	Motility.	Grams stain.	Liquefaction of gelatine.	Litmus milk.	Acidity in litmus whey.	Reduction of nitrates.	Inosit.
1		+	–	–	+	–	–	+	+	–	–	+	% ..	+	–
2	B. Acidi lactici (Hüppe)	+	–	–	+	–	–	+	–	–	–	+	23	+	–
3	B. Levans	+	–	–	–	+	+	–	+	–	+	+	14	+	–
4	B. Grunthal B. Sulcatus gasiformans B. Castellus	+	–	–	–	–	–	+	+	–	–	+	20	+	–
5	B. Vesiculosus	+	–	–	–	–	–	+	–	–	–	+	25	..	–
6		+	–	–	–	–	+	+	–	–	–	+	..	+	..
7		+	–	–	–	–	–	–	+	–	–	+	..	..	–
8	B. Coli mutabilis (Massini)	+	–	–	–	–	..	–	–	–	–	+	..	..	..
*9		+	–	–	+	–	+	+	–	..	..	..	..	..	..
*10		+	–	–	–	+	–	..	..	..	..	..	..	..	..
33		+	–	+	+	–	–	+	+	–	–	+	..	..	–
34	B. Coli communis	+	–	+	–	–	–	+	+	–	–	+	28	+	–
	B. Cavicidæ (Brieger)	..	..	..	..	..	..	..	..	..	..	..	..	..	..
35	B. Schäfferi	+	–	+	–	–	–	+	–	–	–	+	22	+	–
36		+	–	+	–	–	–	–	+	–	+	+	..	+	–
*37		+	–	+	–	–	+	..	–	..	..	..	..	..	..
*38		+	–	+	+	–	+	+	–	..	..	..	..	..	..
*39		+	–	+	–	+	–	..	+	..	..	..	..	..	..
65	B. Oxytocus perniciosus	+	+	+	+	+	+	+	–	–	+	+	26	+	+
66		+	+	+	+	–	–	+	–	–	–	+	..	+	–
67		+	+	+	+	–	+	–	–	–	–	+	..	+	+
68	B. Pneumoniæ (Fried lander) B. Rhinoscleroma	+	+	+	+	–	–	–	–	–	–	+	14	+	+
69		+	+	+	–	+	+	–	+	–	+	+	..	+	–
70		+	+	+	–	+	–	–	+	–	+	+	..	..	–
71		+	+	+	–	–	–	+	+	–	–	+	..	+	–
72	B. Neapolitanus	+	+	+	–	–	–	+	–	–	–	+	22	+	–
73		+	+	+	–	–	+	–	+	–	+	+	..	+	–
74		+	+	+	–	–	–	–	+	–	–	+	..		..
75		+	+	+	–	–	+	–	–	–	–	+	..	..	+
97		+	+	–	+	+	+	+	–	–	+	+	..	+	–
98		+	+	–	+	+	+	–	–	–	–	+	..	+	+
99		+	+	–	+	+	–	–	–	–	–	+	..	+	+
100		+	+	–	+	–	–	+	+	–	–	+	..	..	..
101		+	+	–	+	–	–	+	–	–	–	+	..	+	+
102		–	+	–	+	–	+	–	+	–	–	+	..	..	..
103	B. Lactis ærogenes B. Dysentericæ B. Vitulorum B. Capsulatus (Pfeiffer)	+		–	+	–	+	–	+ –	–	–	+	30	+	+
104	B. Gasiformans non-liquefaciens	+	–	–	+	–	+	–	–	–	–	+	12	+	–
105		+	–	–	–	+	+	–	+	–	+	+	..	+	–
106		+	–	–	–	–	–	+	+	–	–	+	..	..	–
107	B. Coscoroba	+	–	–	–	–	–	+	–	–	–	+	25	+	–
108	B Cloacæ	+	–	–	–	–	+	–	+	–	+	+	20	+	–
109		+	–	–	–	–	–	–	+	–	–	+	..		–
*110		+	–	–	–	+	–	..	..	..	..	..	..	..	..

APPENDIX A.

DETAILED DESCRIPTION OF THE METHOD OF ANALYSES EMPLOYED.

FOR the benefit of those who may be engaged in actual water analysis we consider it advisable to give a brief description of the actual details of the method employed in this work. All samples for bacteriological purposes are taken by a special messenger who has been carefully trained in the technique. He is supplied with sterile bottles of 250 c.c. capacity. These are carefully wrapped up in brown paper, a piece of string is attached to the neck of each (in case the bottle is required for taking samples from rivers), and the bottle, paper, string and all, are sterilised in the autoclave. The messenger is also supplied with sufficient ice to enable him to keep the samples cool during transit to the laboratory. This is a very necessary precaution. Samples sent by train, packed in ice, are subject to so many accidental delays that we find that for bacteriological work in India, at any rate, this method of transit is not sufficiently satisfactory to be practicable. Travelling is distinctly cheap in India, and the expense incidental to collecting the samples is not very great, provided a regular programme of work for the year is prepared and adhered to.

On arrival at the laboratory three separate series of bacteriological analyses are at once started, the bottles having been well shaken.

These are (*i*) total colonies count in 1 c.c. of water, and (*ii*) inoculating of tubes of MacConkey's bile salt broth with various quantities of water: (*iii*) 20 c.c. is centrifugalized in order to test for streptococci.

(1) Total colonies in 1 c.c. A count of the total colonies in 1 c.c. of water is made on agar. The medium used for this is very carefully prepared, so as to get each brew of the same reaction and strength. Lemco ½ per cent., Witte's peptone 1

per cent., is used as the nutrient medium, with 2 per cent. of agar, and a standard of acidity of 10 to phenol phthalein is allowed. One c.c. and 1 10 c.c. of a water is taken and run into the melted agar, when it has cooled down to a temperature of 38°C. This is plated out in the usual way in a petries dish, incubated at 37°C for 48 hours, and all colonies that are visible to the naked eye are counted. These results, though they are extremely interesting, especially when comparing one sample of the same supply with that of another, cannot be compared with total counts obtained on gelatine. The climate of this country renders gelatine as a solid medium extremely difficult to work with. The incubator that will maintain a temperature of 19° centigrade constantly, through all the vagaries of the Indian climate on the plains, does not appear to have been designed yet. Obviously, therefore, it is better to have a method of doing total colony counts that is equally satisfactory in both hot and cold weather, and, provided that care be taken with the manufacture of the solid agar media, so that each batch is as nearly as possible like the previous one, the results obtained are valuable, and can be compared with each other with a reasonable expectation of concordance.

In the earlier chapters the advantages and disadvantages of glucose and lactose in the first stages of the separation of fæcal organism have been discussed. For all ordinary samples we recommend lactose, but, when it is desired to ascertain the preponderance of the group glucose + lactose – both media have to be used.

(2) Bile salt broth.—Varying quantities of the water are put into these tubes. One tube receives 20 c.c. of the water ; 2, 10 c.c. ; 4, 5 c.c. ; 5, 1 c.c. ; 5, ·1 c.c. ; and 5, ·01 c.c. (for these first three tubes a concentrated broth is used, so that the dilution, after the addition of the water, is kept approximately equal). It will be observed that the first three series each make up to 20 c.c., so that, if there is only one tube in the first seven of these which gives the reaction, it means that fæcal bacilli are present only in 60 c.c. (*i.e.*, 3 twenties). This arrangement of obtaining a result in 60 c.c. has obvious advantages. It gives considerable information as to the number of

these organisms present in this quantity of water. In the latter 3 groups it is, of course, impossible to keep the total quantity of water used the same. Therefore, for purposes of recording results, we consider that there are fæcal bacilli in 1 c.c., if 3 out of the 5 tubes give the reaction. Precisely the same applies to the ·1 c.c. and ·01 c.c. groups. This number of tubes of bile salt broth has given very satisfactory results. It is economical on the whole, and it certainly gives sufficient detail of the quantity of fæcal bacilli present in any given sample. The total number of tube per sample used is 22, and we do not consider that it would be advisable to cut this down to any great extent. If, however, saving of media is of great importance, 3 tubes to each of which 5 c.c., 1 c.c., ·1 c.c., and ·01 c.c. of water are added, might be used ; and if 2 out of 3 in a group gave the reaction, fæcal bacilli might be considered as present in that group. Only tubes that give acid and gas are counted as giving the reaction. The bile salt broth tubes are incubated at 37°C ; 42°C is not satisfactory as some attenuated specimens of bacteria fail to ferment a carbohydrate at this temperature, but succeed at 37°C.

The next step is the separation of the various bacilli that have grown in the bile salt broth, and their identification by passing them through the sugars. The method of doing this is as follows, and it is necessary to carry out these details with considerable care, otherwise great variety in the results will be obtained, owing to the possibility of causing too great acidity in the bile salt medium. Practically all of the bile salt broth tubes that have had water added, will show acid and gas after about 18 or 24 hours' incubation. For sub-culturing the bacilli present a tube containing a large quantity of the water is taken. In actual practice we take one tube containing always 20 c.c. of the water, 18 hours after it has been inoculated. If tubes which have received smaller quantities of water are taken for this purpose, there is a danger that perhaps a single fæcal bacilli may have been added to the tube, and in that case a pure culture of this bacillus is obtained, this cannot represent the bacteriological flora of the water as a whole, whereas the growth from 20 c.c. is much more likely to do so. Further, if the tube selected for sub-culture is allowed to remain in the incubator more than about 24 hours (which is always sufficiently long, provided the bacilli in the quantity taken are fairly numerous), the increasing acidity of the medium due to the fermentation of the lactose kills off the organisms present. Consequently, it is better to begin the work of sub-culturing as soon as the reaction has declared itself, so as to keep the numerical relation of the various bacilli, originally present in the water, as little altered as possible.

Having selected the tube for the sub-culture of the organism, one loopful of the bile salt broth is taken out from the tube and put straight into 10 c.c. of sterile water (tube 1) and well stirred round ; with the same loop a loopful of this dilution is carried into another 10 c.c. of sterile water (tube 2), and so on to a third (tube 3). A loopful from tubes 2 and 3 is put on the centre of a plate containing solid medium and spread out with a sterile glass rod.

The solid medium that is used is a bile salt neutral red lactose agar. The formula for this will be found in Appendix C. This medium gives most excellent results. The presence of the bile salt and the neutral red both tend to retard the growth of moulds and air organisms, so that it does not rapidly go bad in a warm climate. The medium is also particularly easy to make. It is stored in the laboratory in test tubes, melted and poured into plates 24 hours before use. It is necessary to put the plates into the incubator upside down for 24 hours, so as to get the surface of the medium nice and dry, otherwise the colonies become confluent, and the plates are useless. As already stated a loopful from the diluted bile salt broth (tubes 2 and 3) is placed in the centre of these plates and wiped over the entire surface. The plates are incubated at a temperature of 37°C for 24 or 48 hours. The method of dilution given above usually produces plates containing about 10 to 20 colonies per plate from tube 3, and something between 50 and 200 from tube 2, the object being to get a plate which does not contain more than about 20 colonies, for if this is done successfully, it becomes a very easy matter to take either all the colonies, or half of them, for putting through the sugars, and by this means it obviates all selection, so reducing the "speculative venture" as Houston rightly describes this selection, to a minimum.* The colonies present a very great variety of appearances on this solid medium. Some are bright red ; some have a red centre with a pale rim ; others are white, or yellow, and some have a transparent look. The red colonies usually cause a precipitation of bile acid in the solid medium producing a haze round the colony. The appearance of any particular colony on the medium is of little or no use for identifying the bacillus present, very little reliance can be placed on the naked eye appearance. Two colonies very different in appearance, the one bright red with haze of precipitated bile salt round it, the other pale yellow, may on further investigation turn out to be the same organism. The reason for this is probably due to the fact that acid and alkali may be both produced by colonies at different times, sometimes the one predominates, and sometimes the other.

Having obtained a good plate with, say, 10 or 12 discrete colonies on it, each separate colony is now put through the various sugar reactions. If the colonies are small, and are crowded together, or if, as sometimes happens, instead of getting a plate containing 10 colonies, it contains 100, it is advisable to pick off 10 or 12 colonies, while they are small, and inoculate agar slope tubes from each one. In doing this care should be taken not to select 10 colonies from all over the plate, otherwise the tendencies to get them all of one kind is natural, and the " speculative venture " factor comes in, but it is better to mark off a segment of the plate and take all out of the part marked. If, on the other hand, the plates have been successful, and colonies are separate, it is possible to inoculate the 6 tubes from each colony, without passing through the stage of growing it separately on agar. If this latter method is to be carried out the first thing to do is to number the colonies on the back of the plate with a pencil, otherwise confusion will occur. Six separate tubes containing the various materials are inoculated from each colony. The safest and quickest method of carrying out this operation is to scoop up the whole colony with a loop, put the growth in a small test tube (3″ by ½″) containing a few drops of sterile water, mix well, and place 6 sterile iron wire loops in the mixture—one wire is used to inoculate each of the 6 tubes containing the sugars.

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* Houston says : " The picking out of coli-like colonies for studying in pure culture is, after all, even to the expert, a speculative venture."

The tests that are recommended by MacConkey are saccharose, dulcit, adonit, inulin. Two other tubes are also inoculated, the one a glucose tube for the Vosges and Proskauer's reaction, and a broth tube for the indol reaction and for motility.

The four sugar media are made up with sugar free peptone, bile salt, and neutral red, according to MacConkey's formula. The glucose tube contains neither neutral red nor bile salt. These tubes are placed after inoculation into one rack and are incubated for 48 hours at 37°C. The broth tube, however, is examined for motility after 18 hours' growth of the organism. The reaction in the sugars is considered to have taken place if gas and acid are developed. Gas is not always very apparent. The Vosges and Proskauer reaction is completed by adding a few drops of a very concentrated solution of caustic potash after 48 hours' (or more) incubation. The red colouration nearly always appears within an hour, but in some cases it is delayed. The tubes are, however, kept for 24 hours after the caustic potash has been added. The indol test is done with persulphate of potash and paradimethyl-amido-benzaldehyd. The method of recording the results of these changes in the sugars will be seen from the tables given of the various analyses, all tubes that give a definite reaction are marked as +, those that fail — ; it is very seldom that there is any doubt about the result, but when this does occur an extra 24 hours in the incubator usually settles the matter definitely one way or the other.

These may be taken as the working details of the method used in this book ; the various media will be found in the table given in Appendix C. The method, it will be observed, does not differ materially from all others in daily use, the important improvement being that the organisms, that grow in the bile salt broth, are, by the use of MacConkey's system of passing each organism through certain fermentation tests, separated out into their individual species.

It may be well to discuss the value of some of these tests in a little greater detail:—

(1) The total Colonies on Agar.—This should always form part of the examination. It affords very valuable information regarding the bacilli, other than the lactose fermenters, that are present in a given sample. By comparing the total colonies with the number of lactose fermenters present in the bile salt broth the relation between fæcal and ordinary water organisms is roughly ascertained. The particular value of the total counts is not so much in comparing samples from different parts of a Province, though this is possible, but in comparing the various samples derived from the same source. Here it may be pointed out that as a routine method we usually take 4 or 5 samples from each system; one from the natural water, whether it be well, spring or river; one taken immediately after filtration (whether it be filtered through a gallery or through constructed filter beds); and 2 or 3 samples from the distributary system, and the service reservoir if there be one. We not infrequently find that the number of lactose fermenting organisms in a system varies very little wherever the samples are taken, but the total colonies in 1 c.c. gives us much valuable information as to the efficiency or otherwise of the filtration, the action of the pipes on the water, and the presence or absence of silt in the terminal branches of distributary system. One is perfectly prepared to admit that gelatine as a culture medium is superior to agar for this work, because besides giving all the information agar does, it differentiates between the liquefiers and the non-liquefiers of gelatine; further the colonies of the various bacilli present certain characteristic appearances on this medium. Neither of these advantages can be claimed for agar-agar; but the difficulties in the way of working with gelatine are so great in hot climates, that they seriously interfere with its usefulness.

(2) Vosges and Proskauer Reaction.—The Vosges and Proskauer reaction has been the subject of very considerable controversy. Some regard this test as being very variable, at one time giving the reaction, and at another failing to give it with the same bacillus. Orr in his work on milk says: "The reaction has been given by other organisms than lactis æro-

genes, B. Cloacæ, and Oxytocus perniciosus, more especially by those of the proteus group. It is not given constantly by all bacilli of these groups, although, it is apparently a very constant and excellent confirmatory test for B. Cloacæ and Oxytocus perniciosus. It is not apparently so good a test for B. Lactis ærogenes as stated by MacConkey.'' With this last remark we agree, but for the other organisms the reaction appears to us to be a very satisfactory test, if certain simple precautions, such as, adding a fair quantity of the culture to the glucose broth, incubating for 48 hours or more, are always observed. Some care has to be exercised, as in all similar reactions, for in some instances the colour is rather faint. If, however, a very small quantity (2 or 3 drops) of a saturated solution of caustic potash is used, instead of a large quantity of dilute solution, the amount of fluid in the tube is not increased, and the colour very seldom fails to appear in sufficient quantities to be easily recognisable in two hours. B. Lactis ærogenes loses its power to give the reaction under the influence of sunlight, but recovers it again if it is cultivated in laboratory media.

(3) Motility.—On the subject of motility as a constant characteristic of any organism opinion is again divided. Admitting, for the sake of argument, that motility is a perfectly constant character of a given species of bacillus, in other words, a motile organism possesses cilia and a non-motile one does not, it nevertheless is certain that a bacterium that is really motile, may be found at times with loss of motility which can be easily restored to it by culturing it. If the work of this report had for its object the solution of purely bacteriological problems, then all non-motile organisms would be cultured two or three times in broth in order to ascertain if this characteristic had been temporarily lost. But for routine work this is out of the question, consequently we recommend that motility be carefully looked for once, *viz.*, after 18 hours' cultivation in broth, but that when a negative result is obtained too much importance be not attached to the result, as there can be no doubt that this test is not of the same order of importance as the power to ferment a sugar, or alcohol. We have some experiments which tend to show that an organism loses its power of

motility by the action of the sun, and does not recover it easily by growth in the media enumerated above.

There are one or two practical hints on laboratory organisation, which make the method given above a very simple matter, although the description would seem to indicate that it is most tedious and complicated. For these " tips " we are indebted to Dr. A. C. Houston.

(1) It is recommended to provide oneself with a large number of iron wire loops, and also a similar number of short lengths of iron wire for inoculating various tubes from one colony. These iron wire loops and rods take the place of the usual platinum loop. They can be sterilized in the Autoclave in bunches of a 100 and kept in test tubes ready. A separate loop or rod is used for each bacteriological operation. The saving of time thereby effected is obvious.

(2) Small racks are necessary for holding six test tubes for the six tests described. This should be made very small so as to take up a minimum of room in the incubator ; those used in our laboratory contain 12 test tubes, that is two rows of six.

(3) It is very convenient to use a different coloured wool plug for each of the six test tubes containing the six different sugar media.

(4) For such expensive sugars as dulcit and adonit, we use very small test tubes on the score of economy. Those measuring 3″ by ½″ are quite large enough to give the reaction satisfactorily and their use effects a large saving.

(5) Always have plenty of sterile pipettes, bent rods, etc., ready at hand. This saves a great deal of time and temper. By the use of the coloured wools and by numbering the racks serially, all labelling or even numbering of tubes is avoided.

APPENDIX B.

A FEW REMARKS ON CHEMICAL ANALYSIS OF TROPICAL WATERS.

ALTHOUGH this work is largely taken up with the bacteriology of surface waters in India, for the benefit of those who are engaged in analysing the waters a few points may be briefly referred to, concerning the chemical analysis. From a large number of analyses of surface waters, it may be stated that some slight differences would be noticed between the waters in the East and in the West. By far the most important of these points is the great rarity of nitrates in rivers and lakes. From some hundreds of samples we have proved that nitrates are either entirely absent or only present in very small quantities in lakes and most rivers. So universal is this the case, from one end of India to the other, that there must be some very widespread condition to account for it. It is well-known to all in charge of sewage works that, as a general rule a shower of rain, after a considerable interval of drought, causes nitrates to be found in the sewage, due in all probability to the action of nitrifying organism on the surface of the soil. It is perfectly obvious that this condition does not occur in the East, and probably the explanation is that the sun's rays kill off these particular bacilli that are present on the surface. Boulanger, Massol, Calmette and others have shown that most nitrifying organisms are easily killed at comparatively low temperatures. The temperature of the ground in an ordinary Indian summer is very high, and in all probability causes the death of these bacilli. Ground waters obtained from wells and springs, etc., are, however, quite as rich in nitrates in the East as in the West. Indeed there are in India several districts where nitrates are so plentiful in the soil that a certain caste of people separate it and sell it.

Surface waters as a rule contain a very high albuminoid ammonia figure, much of which is due to vegetable matter. In all probability the absence of the nitrifying power on the surface layers of the soil partly accounts for the higher figure in the albuminoid ammonia, so that there is no doubt whatever that the permissible limit of albuminoid ammonia should be much higher in the East than in the West. Thus quite a large number of well-sunned and pure lake waters would be condemned on their high albuminoid ammonia figure, whereas certain shallow wells, whose bacteriological analysis shows that they contain a large number of most undesirable fæcal bacilli, would be passed because the free and saline ammonia are low, and the nitrates high. Chemical analysis in a tropical water, particularly as regards organic nitrogen, is not of very much use by itself, and if possible water should never be judged on this alone. Chemical analysis however should always be carried out in order to ascertain the mineral impurities. Many of the waters in India, particularly those from wells and springs, are extremely hard. The Madras well waters seldom contain less than 25 parts per 100,000 and many others as high as 80 parts per 100,000 of total hardness, much of which is temporary. Apparently the natives of India are not easily upset by this degree of hardness, though many Europeans take time to get accustomed to it.

The surface waters are usually soft in the rainy weather, but on account of the amount of subsoil drainage which goes on into the rivers in the dry weather, the hardness not infrequently greatly increases at this time. For instance, the hardness of the water of the Hooghly at Calcutta in the rains is about 2 to 3 parts per 100,000, but it rises to 18° in the dry weather. These are the most important points in connection with the chemistry of surface water.

APPENDIX C.

LIST OF MEDIA USED IN THE WORK DESCRIBED.

I. (Stock solution A).—Bile salt glucose peptone neutral red broth—

Peptone	60 grms.
Sodium taurocholate	15 ,,
Glucose	15 ,,
Water	1 litre.
Neutral red	10 c.c. of 5 per cent. solution.
Normal sodium Carbonate solution	Till neutralised as indicated by the neutral red.

(B Solution)—

2 parts of A solution + 1 part of water.

(C Solution)—

1 part of A solution + 1 part of water.

(D Solution)—

1 part of A solution + 2 parts of water.

II. Bile salt lactose peptone neutral red broth—

Same as above, lactose being substituted for glucose.

III. Bile salt lactose peptone agar—

Peptone	20 grms.
Sodium taurocholate	5 ,,
Lactose	10 ,,
Agar	20 ,,
Water	1 litre.
Neutral red	10 c.c. of 5 per cent. solution.
Normal sodium Carbonate solution	Till neutral point is reached as indicated by the neutral red.

IV. Nutrient agar—

Agar	20 grms.
Lemco	5 ,,
Peptone	10 ,,
Sodium chloride	5 ,,
Water	1 litre.

Standardised to + 10 using normal sodium carbonate solution.

V. Nutrient broth—

Peptone	10 grms.
Sodium chloride	5 ,,
Lemco	5 ,,
Water	1 litre.

Standardised to + 10 using normal solution of sodium carbonate.

VI. Bile salt peptone neutral red sugar medium—

Peptone	10 grms.
Bile salt	5 ,,
Sugar	5 ,,
Water	1 litre.
Neutral red	5 c.c. of 5 per cent. solution.

To prepare different sugars, the particular sugar is substituted in the column of sugar in the above solution.

The following sugar media are made—

1. Saccharose.
2. Dulcite.
3. Adonite.
4. Inulin.

VII. Glucose medium for Vosges and Prosk. reaction—

Peptone	10 grms.
Glucose	5 ,,
Water	1 litre.

VIII. Strong peptone solution for vibrios—

Peptone	100 grms.
Sodium chloride	50 ,,
Water	850 ,,

10 c.c. of this in 90 c.c. of suspected water converts the latter into 1 per cent. peptone water.

Thacker, Spink & Co., 6 Mangoe Lane, Calcutta.